

Figure 1

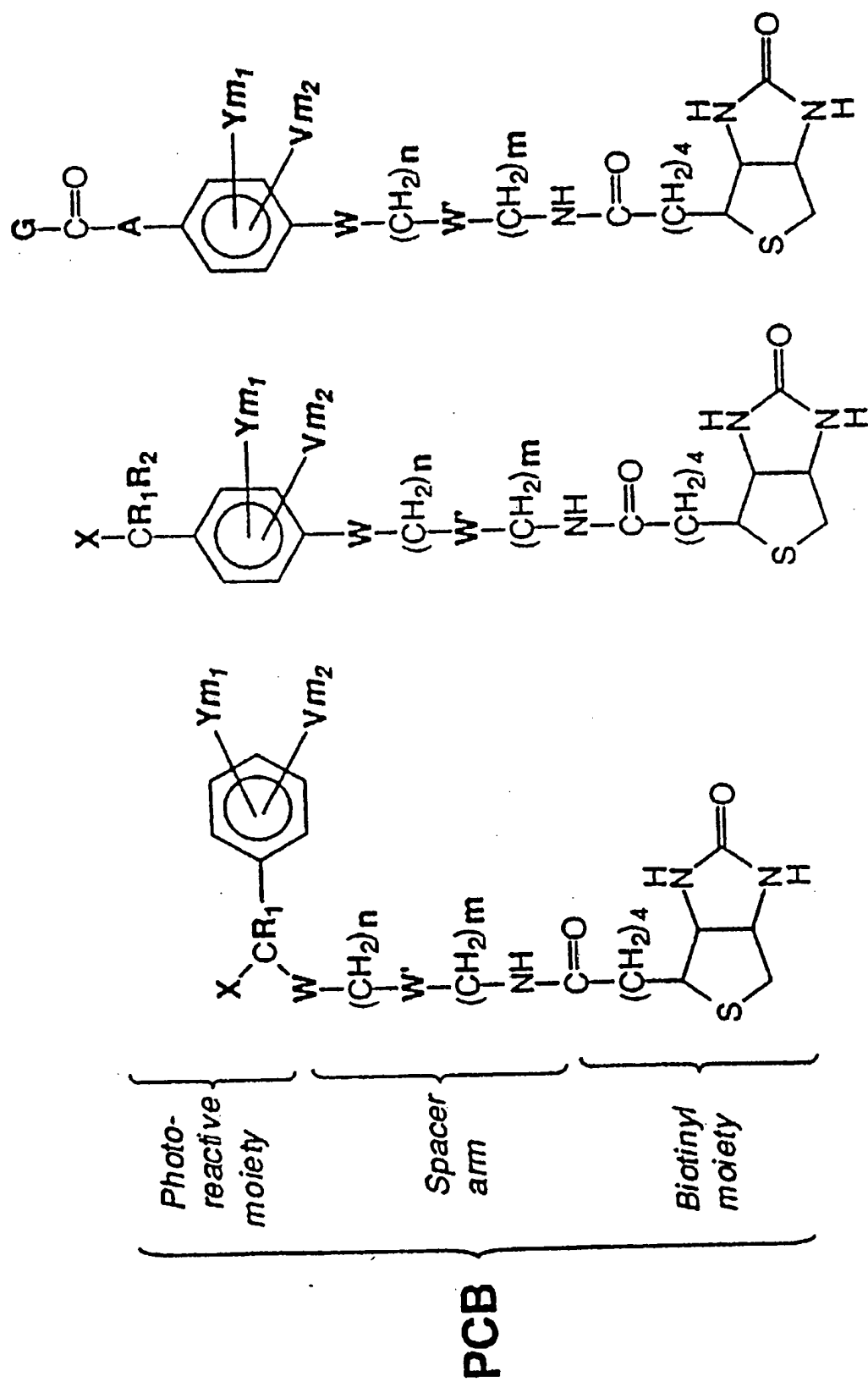


Figure 2

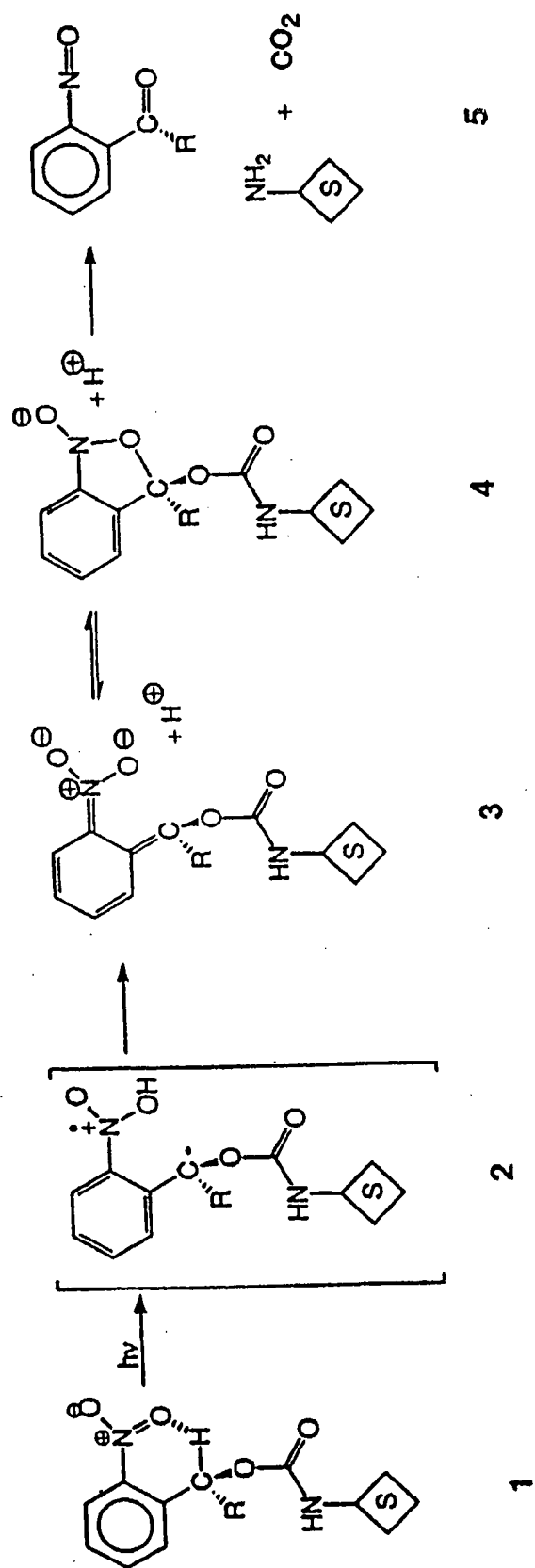


Figure 3

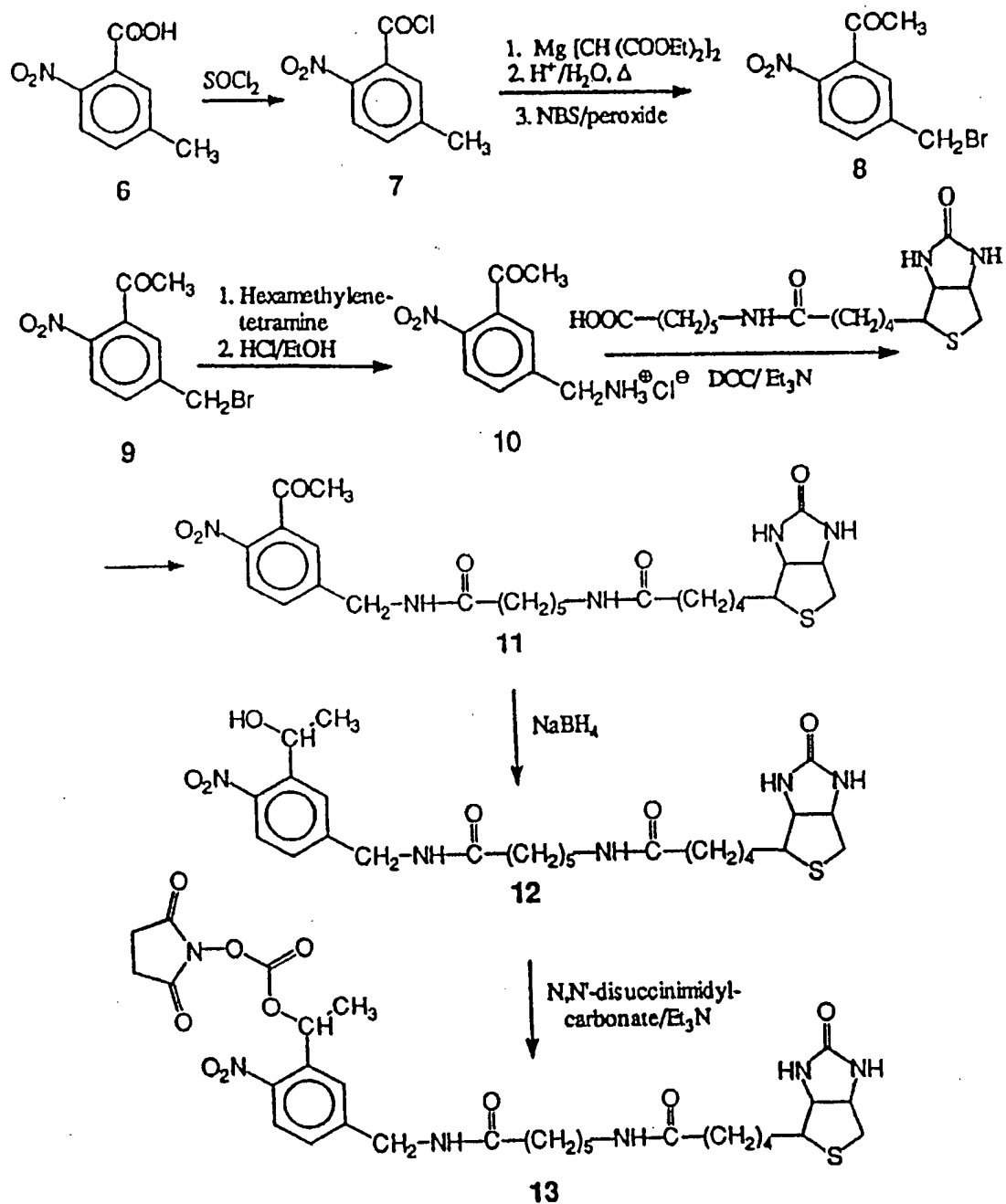


Figure 4

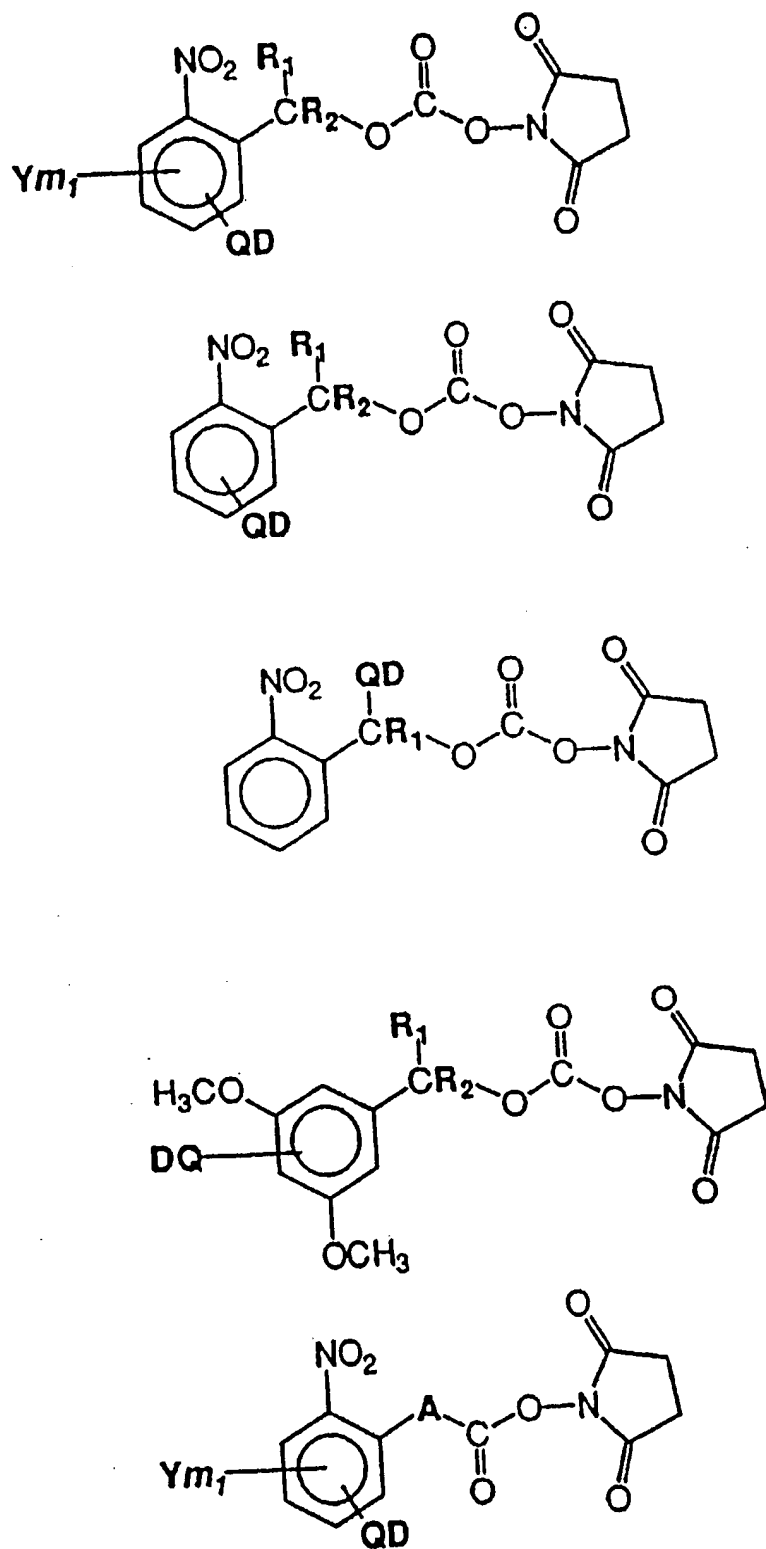


Figure 5

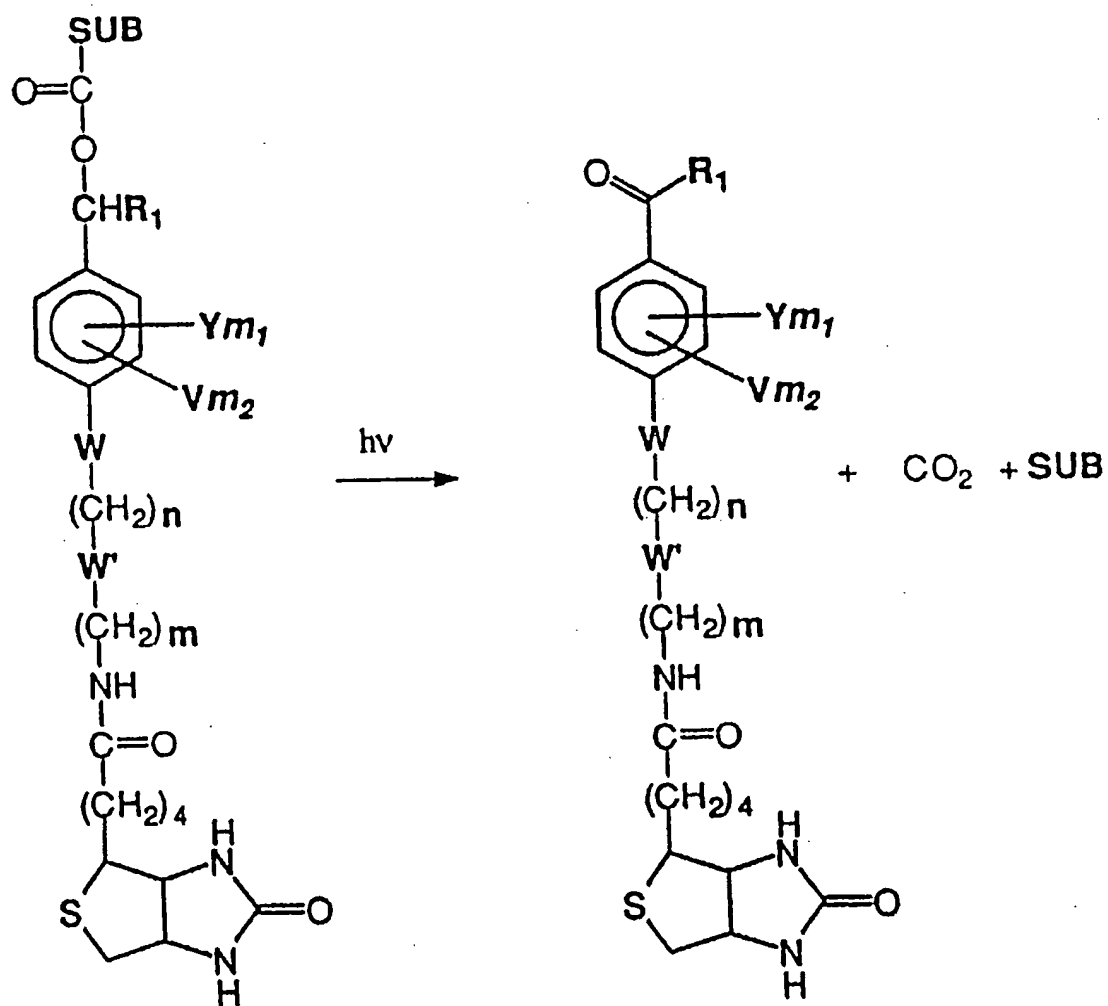


Figure 6

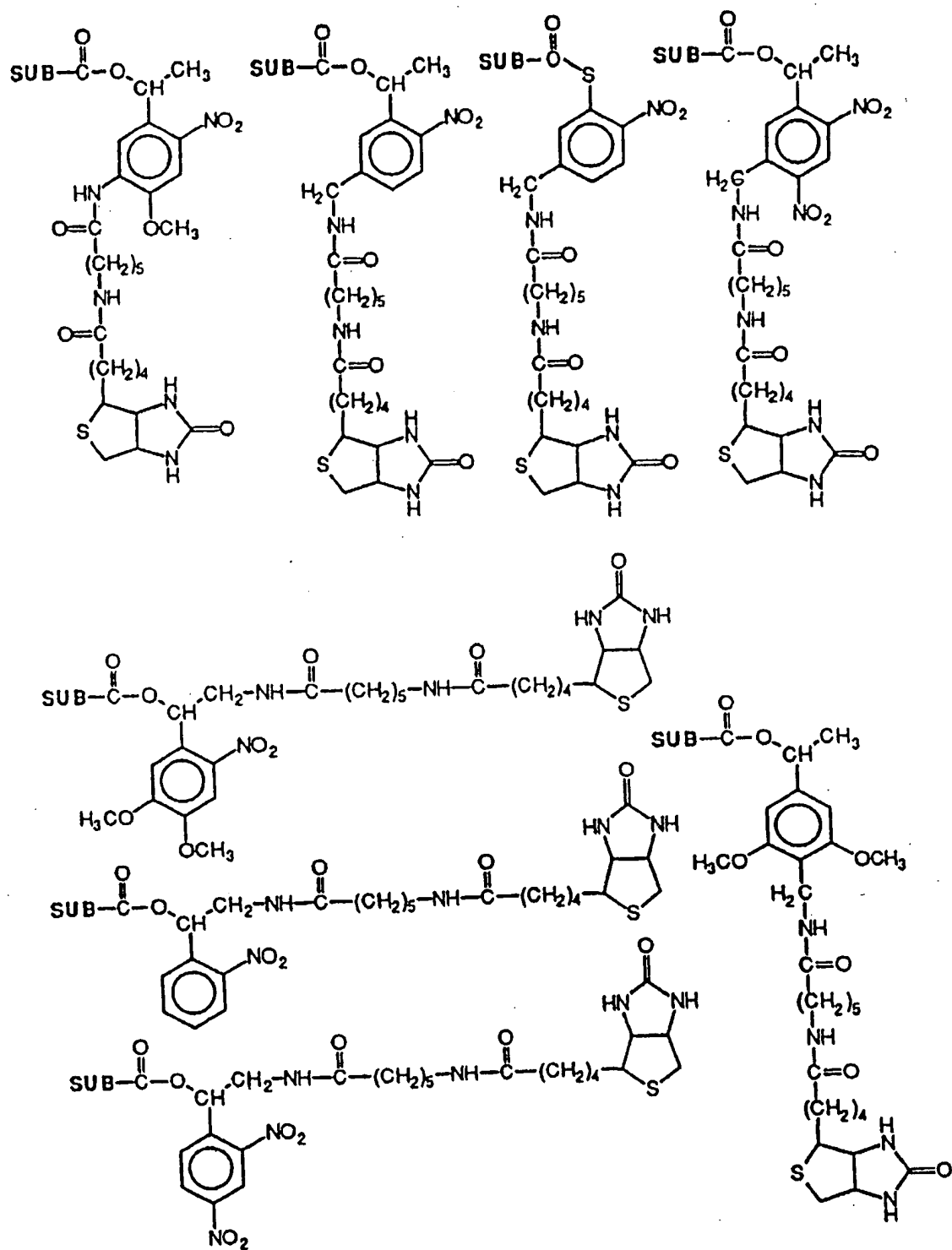


Figure 7

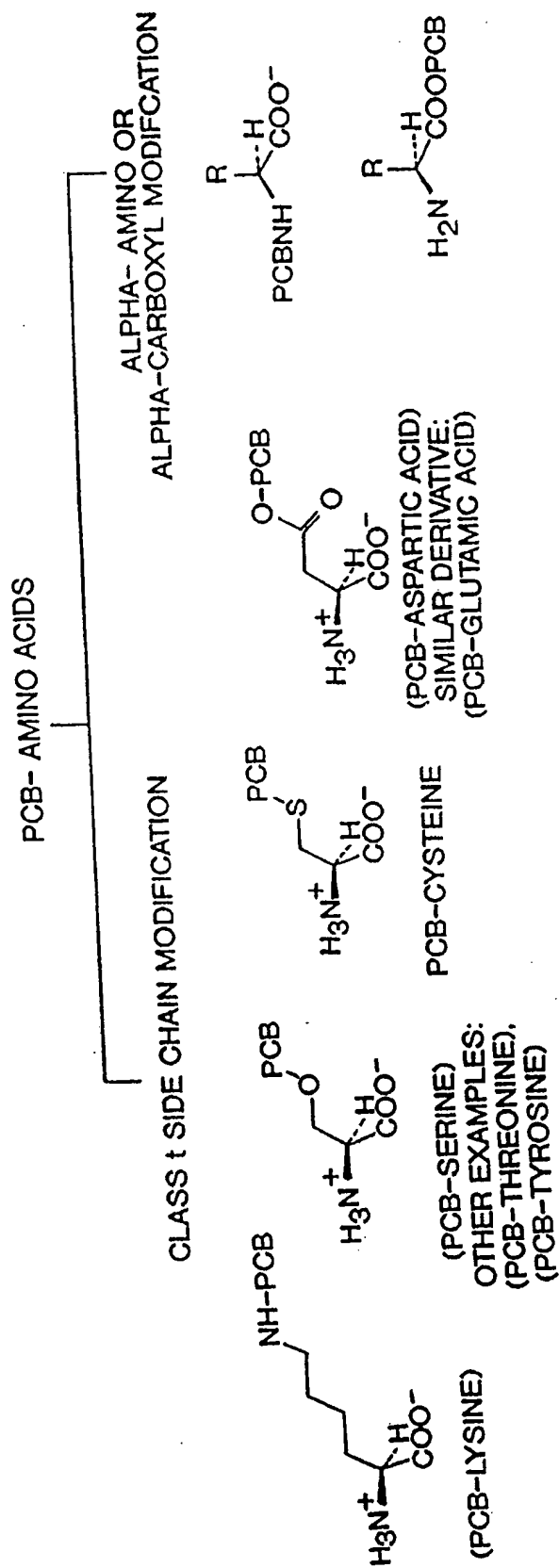
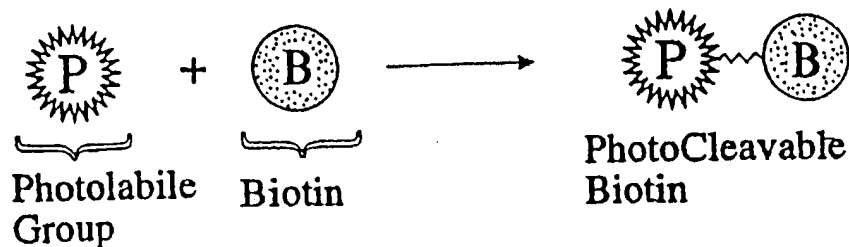
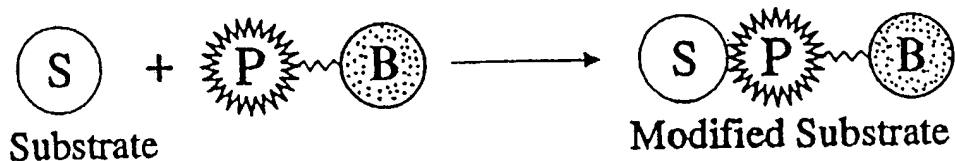


Figure 8

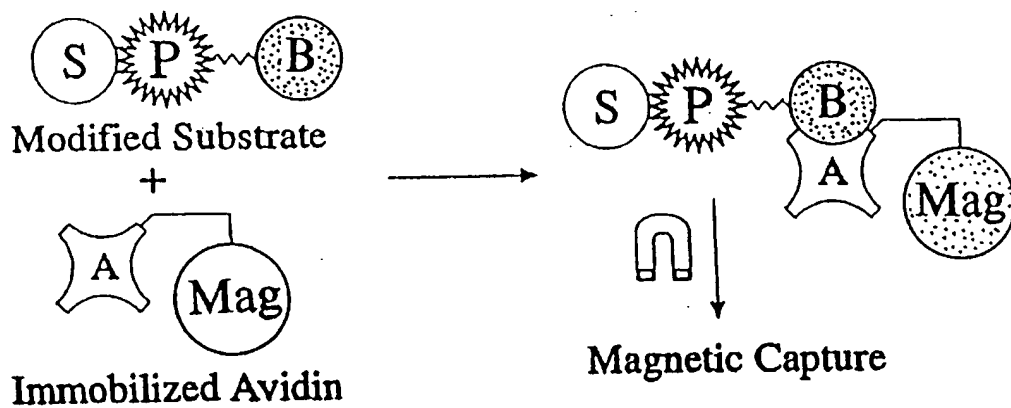
Step 1: Synthesis of Photocleavable Biotin



Step 2: Modification of the substrate by Photocleavable Biotin



Step 3: Isolation of the Modified Substrate using Avidin



Step 4: Detachment of Pure Substrate

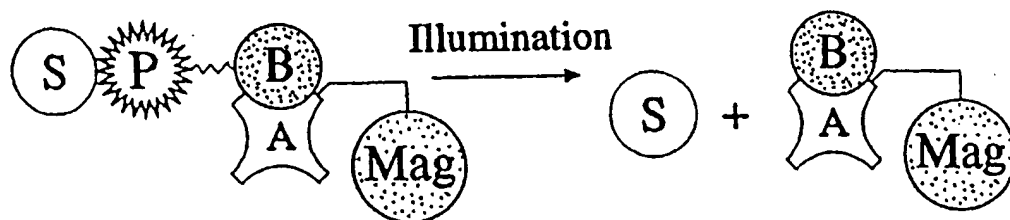


Figure 9

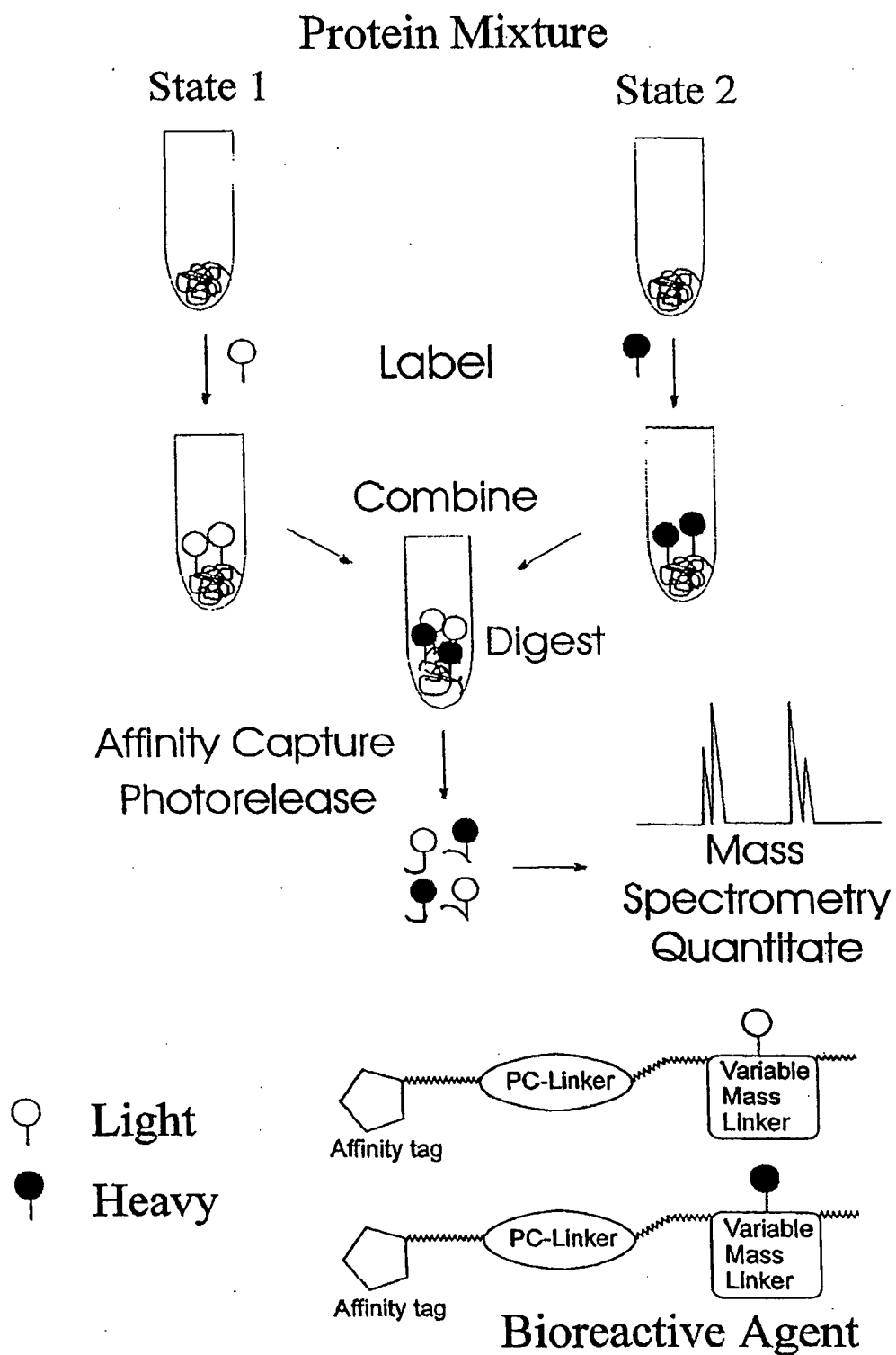


Figure 10

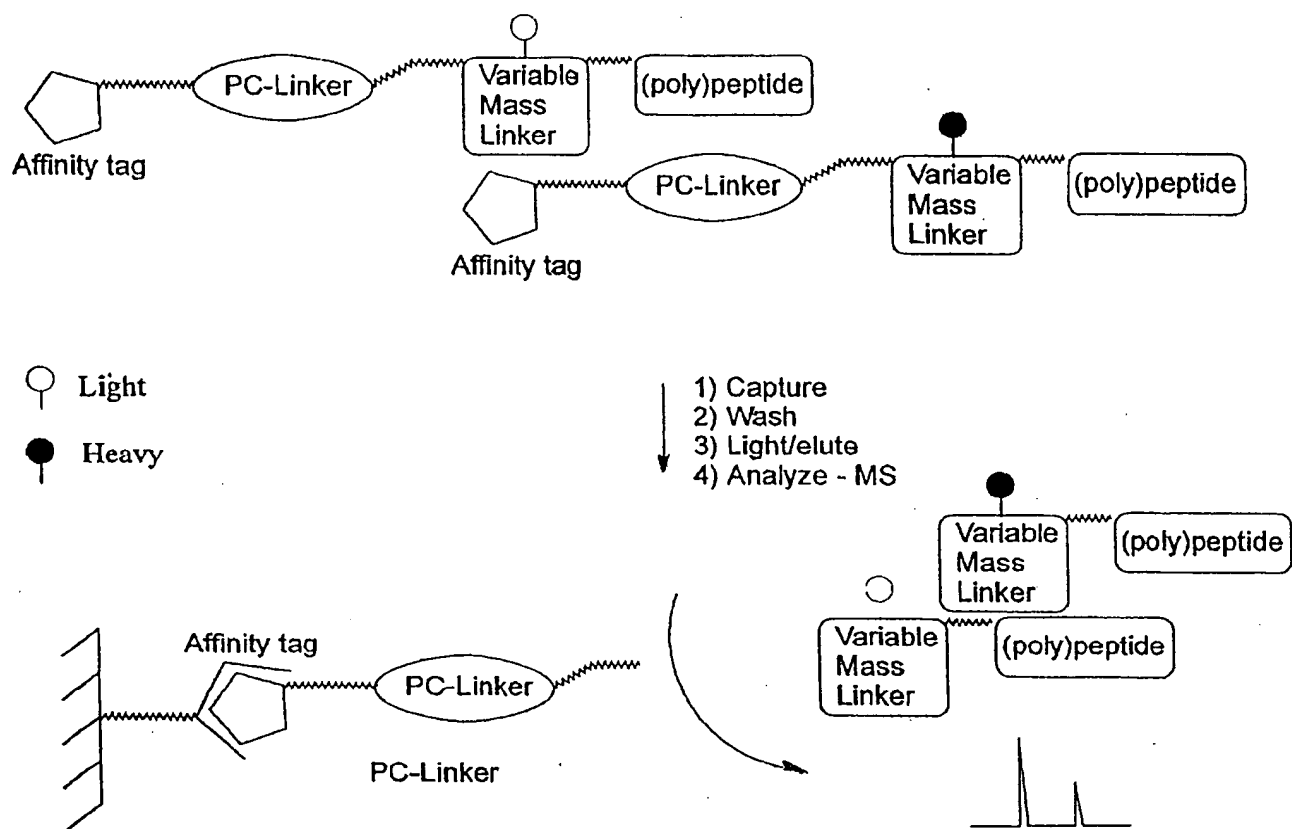


Figure 11

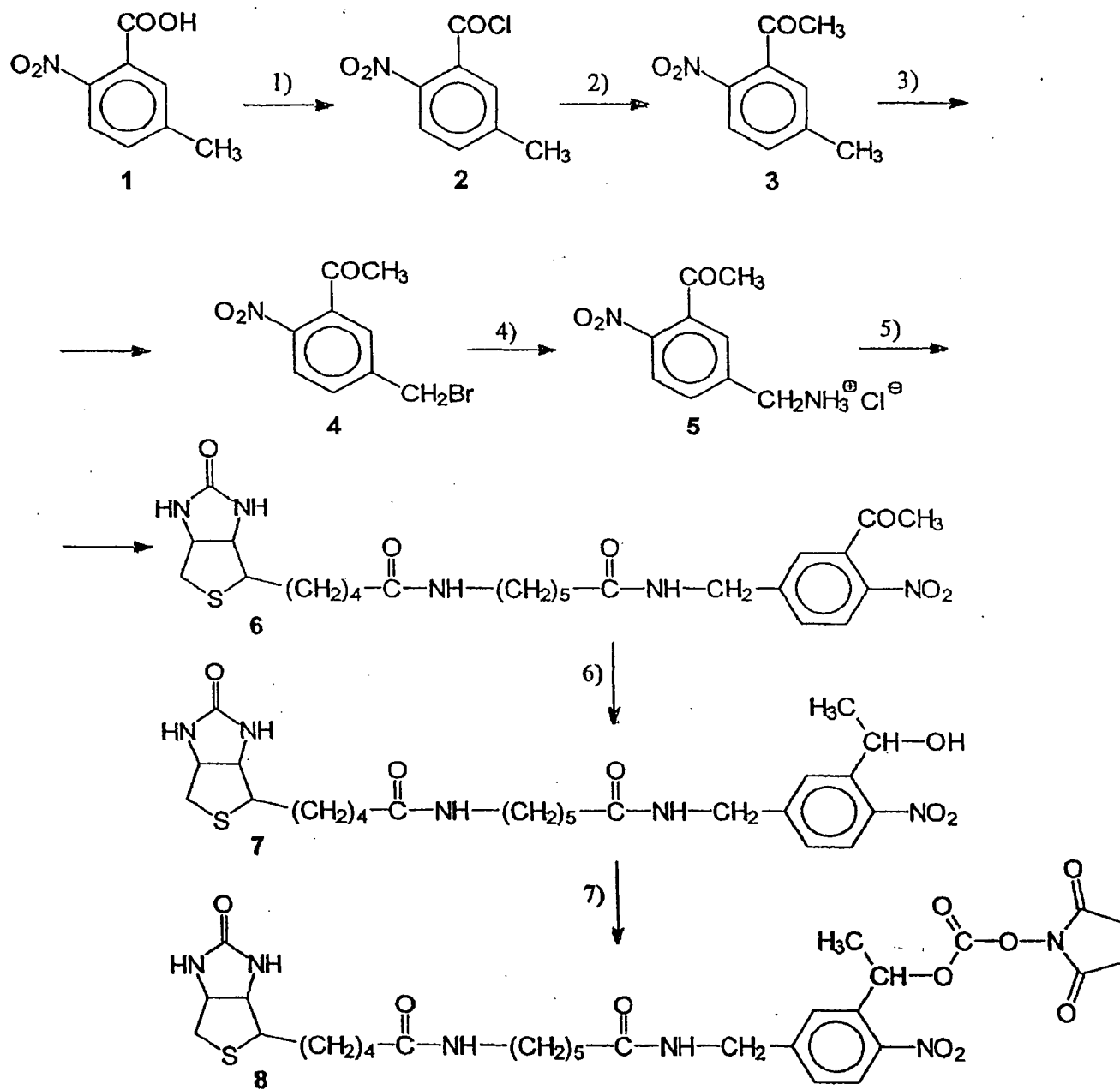


Figure 12



Figure 13

Figure 14A

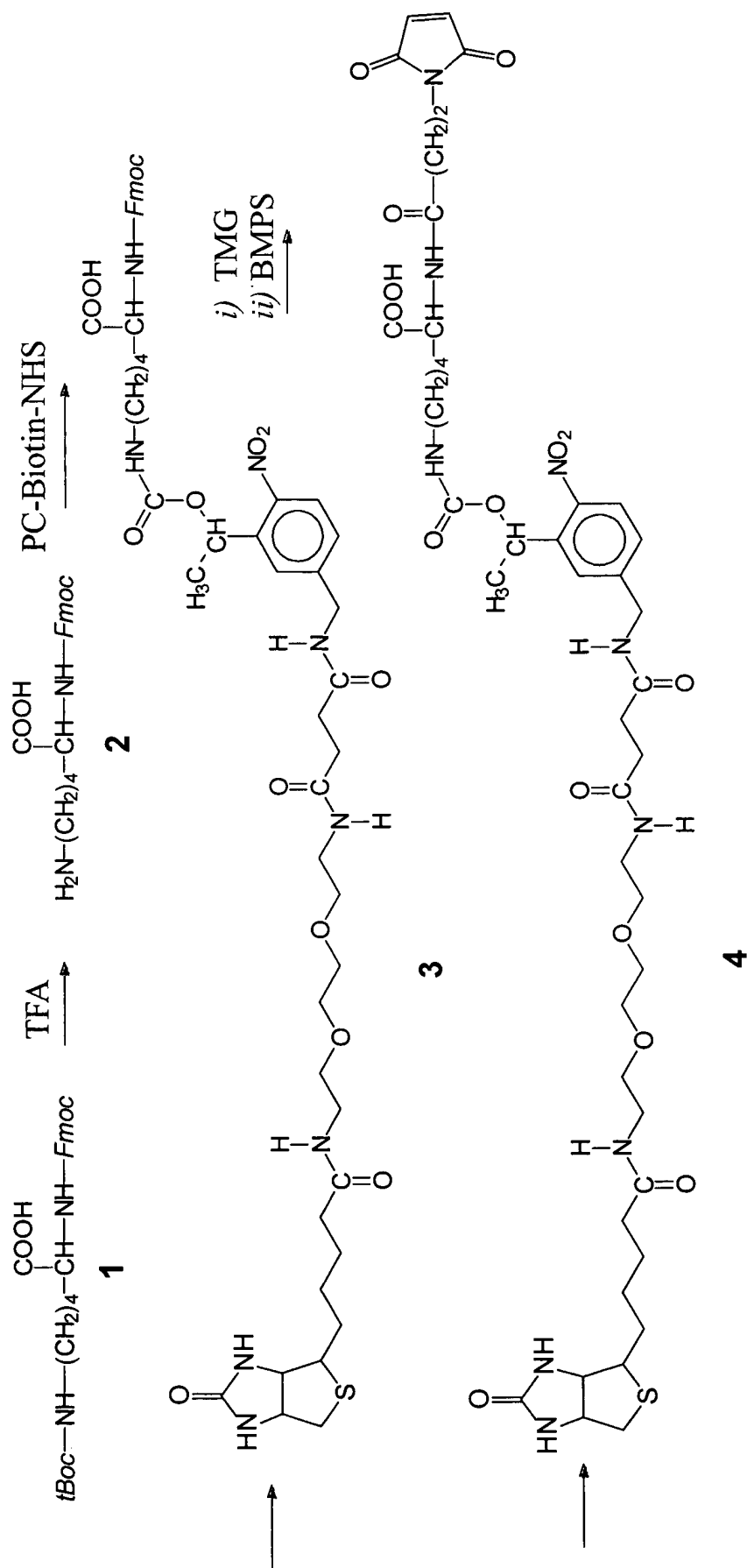


Figure 14B

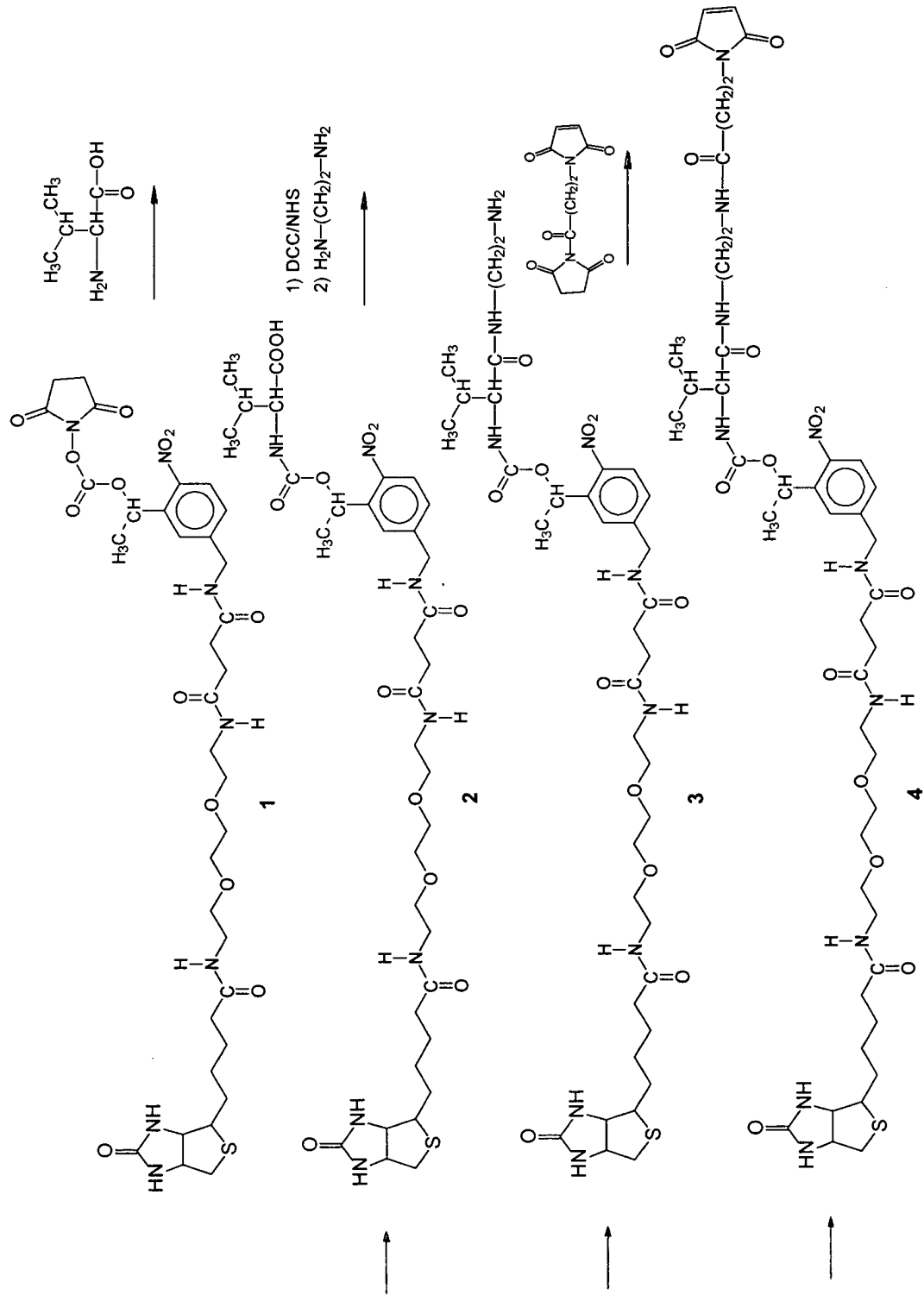


Figure 14C

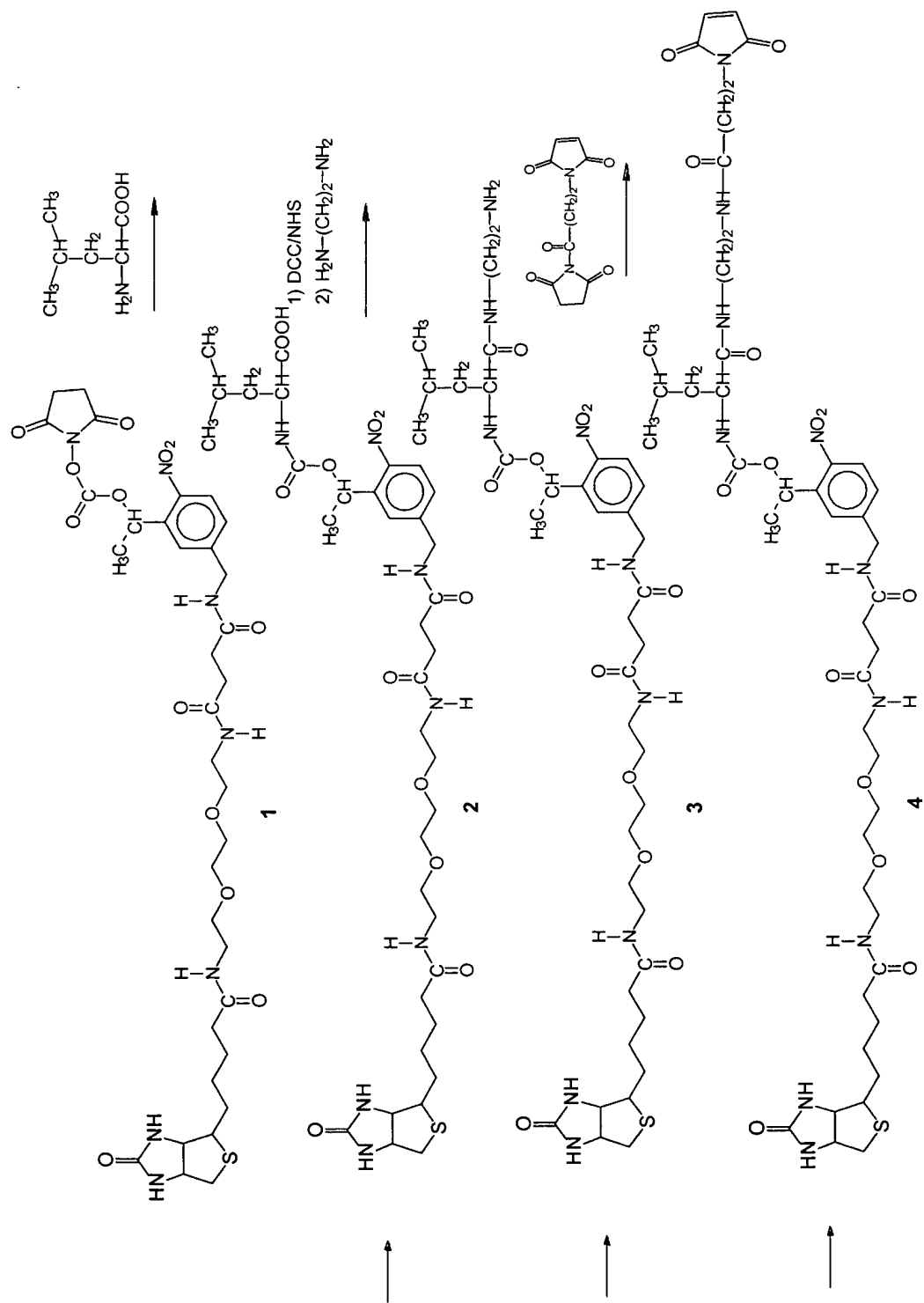


Figure 14D

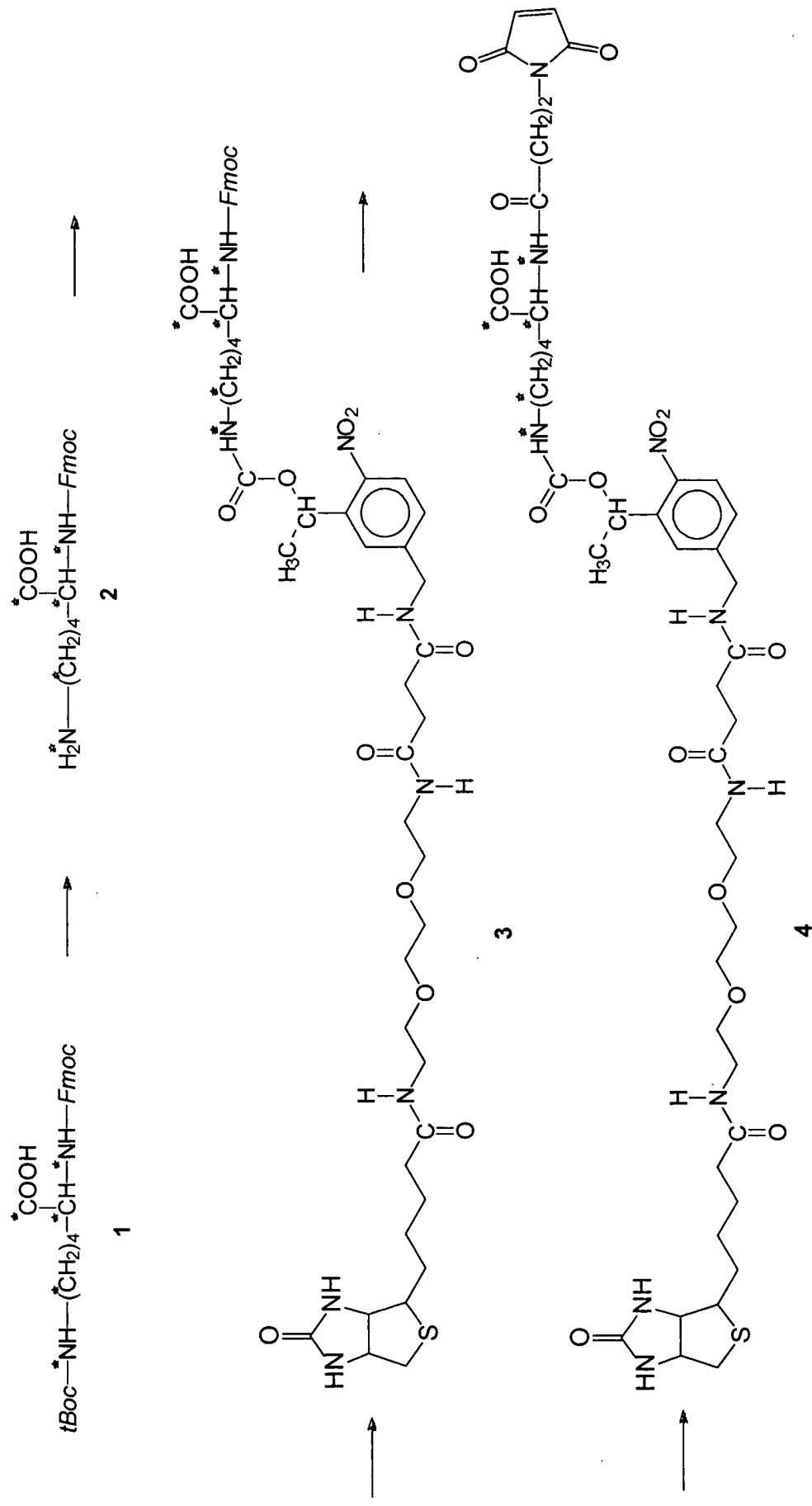


Figure 14E

PC-ICAT Reagent	Reactive Group (Reactivity)	Mass change (after cleavage)
<div data-bbox="516 1644 565 1850">PC-ICAT #1</div> <div data-bbox="526 989 724 1770"> $\text{PC-Biotin} \text{---} \text{HN} \text{---} \boxed{(\text{CH}_2)_4 \text{---} \text{CH} \text{---} \text{NH} \text{---} \text{C}(=\text{O}) \text{---} (\text{CH}_2)_2 \text{---} \text{N} \text{---} \text{Maleimide}}$ </div>	Maleimide (Sulphydryls)	297.3
<div data-bbox="800 1644 849 1850">PC-ICAT #2</div> <div data-bbox="812 1125 932 1770"> $\text{PC-Biotin} \text{---} \text{HN} \text{---} \boxed{(\text{CH}_2)_4 \text{---} \text{CH} \text{---} \text{NH} \text{---} \text{C}(=\text{O}) \text{---} \text{CH}_2 \text{I}}$ </div>	Iodacetamide (Sulphydryls)	186.2
<div data-bbox="1027 1644 1076 1850">PC-ICAT #3</div> <div data-bbox="1037 905 1227 1770"> $\text{PC-Biotin} \text{---} \text{HN} \text{---} \boxed{(\text{CH}_2)_4 \text{---} \text{CH} \text{---} \text{NH} \text{---} \text{C}(=\text{O}) \text{---} \text{O} \text{---} \text{NHS ester}}$ </div>	NHS ester (Amines)	242.3

Figure 14F

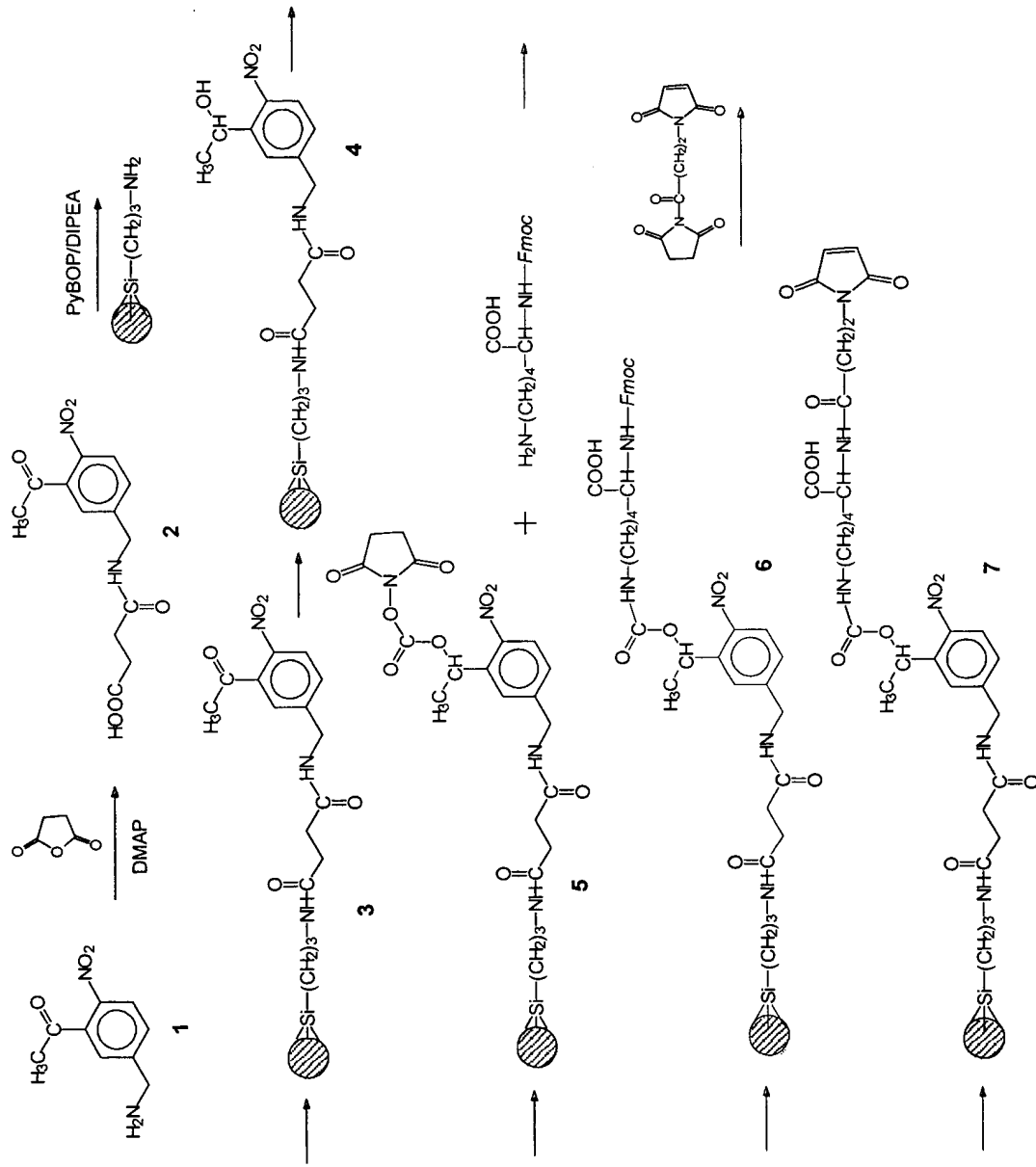


Figure 15

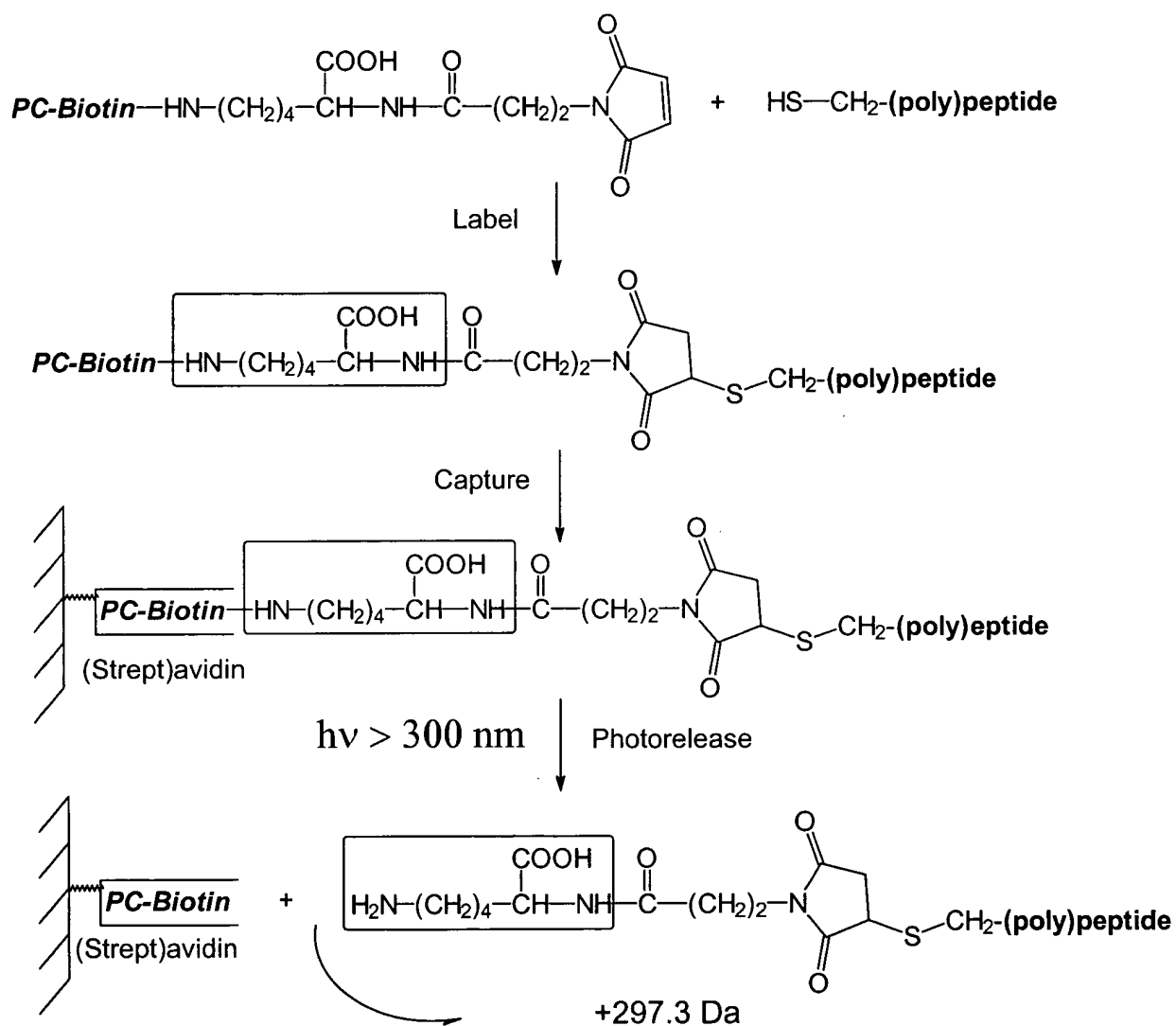


Figure 16

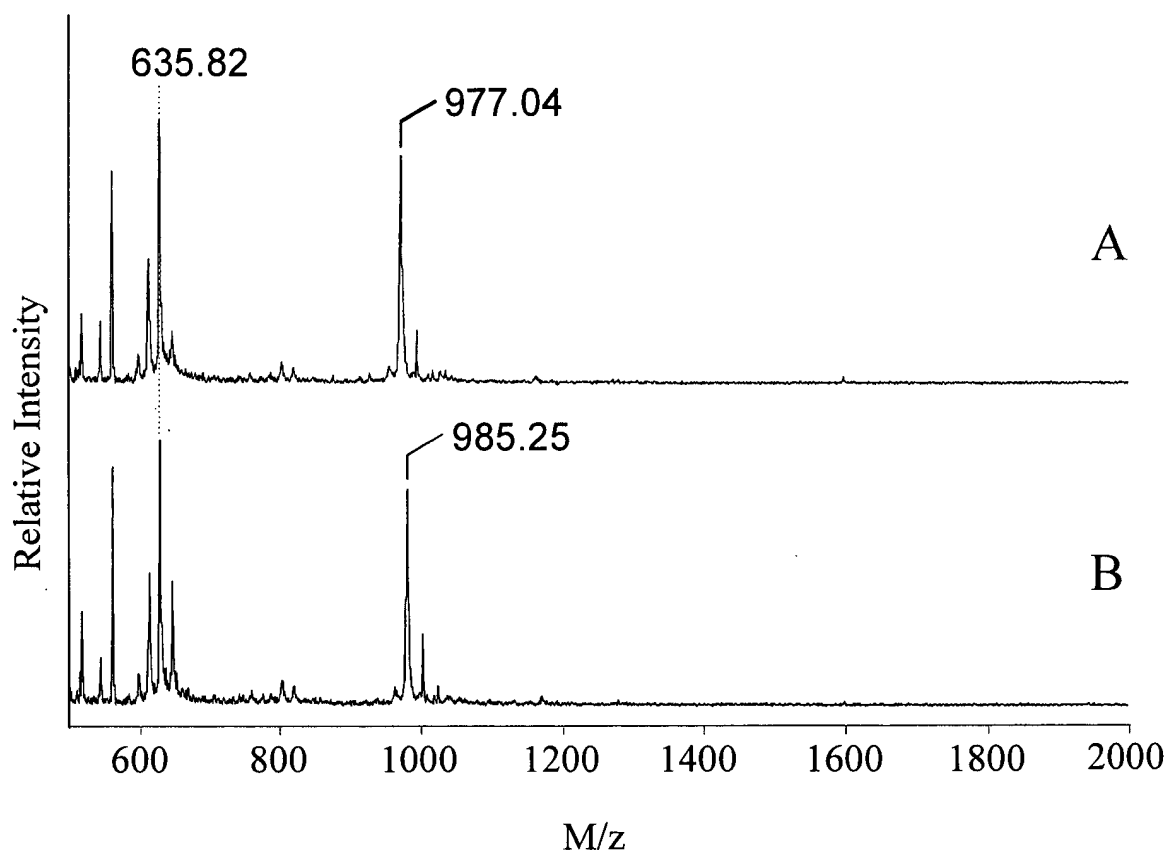


Figure 17

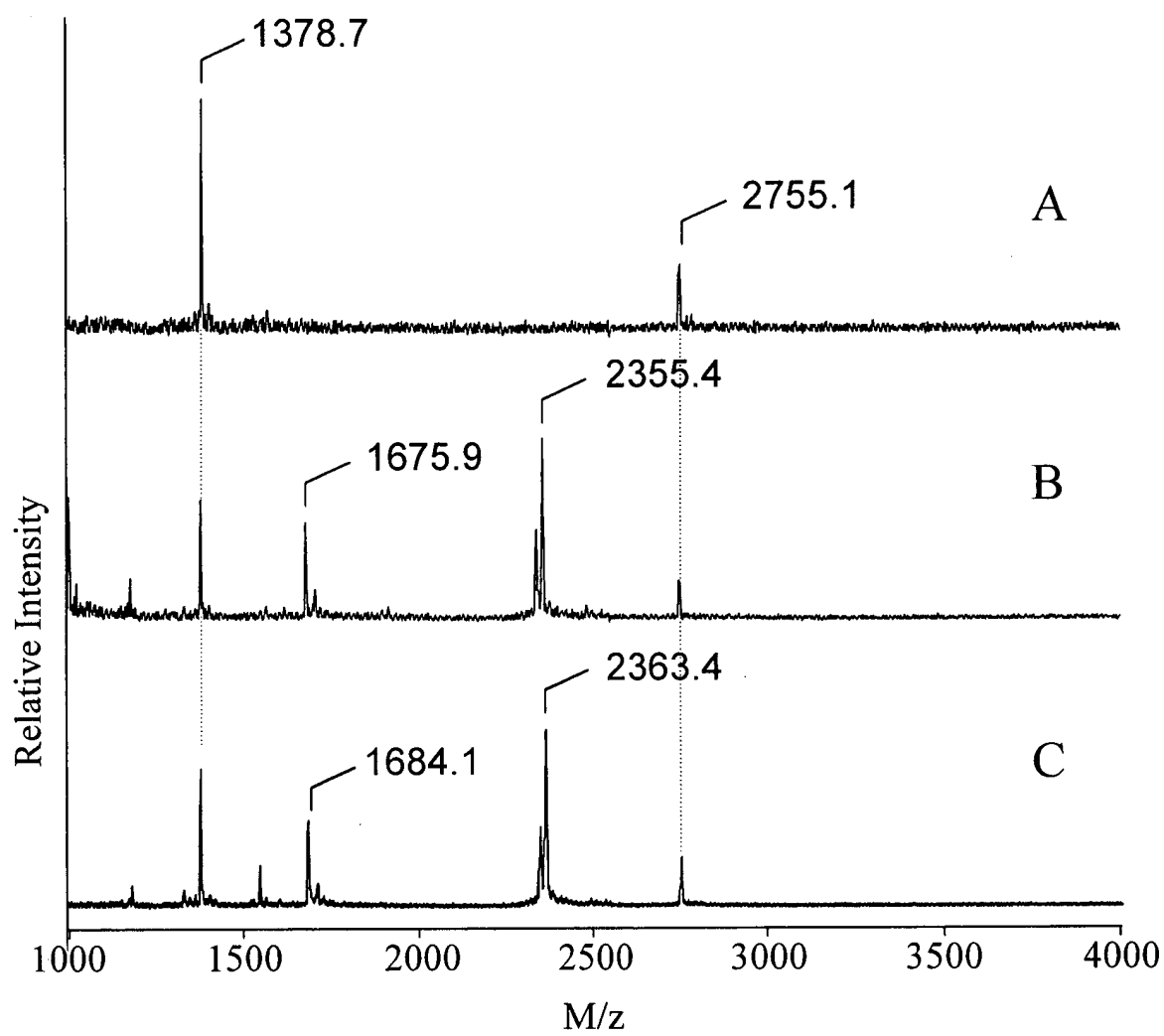


Figure 18

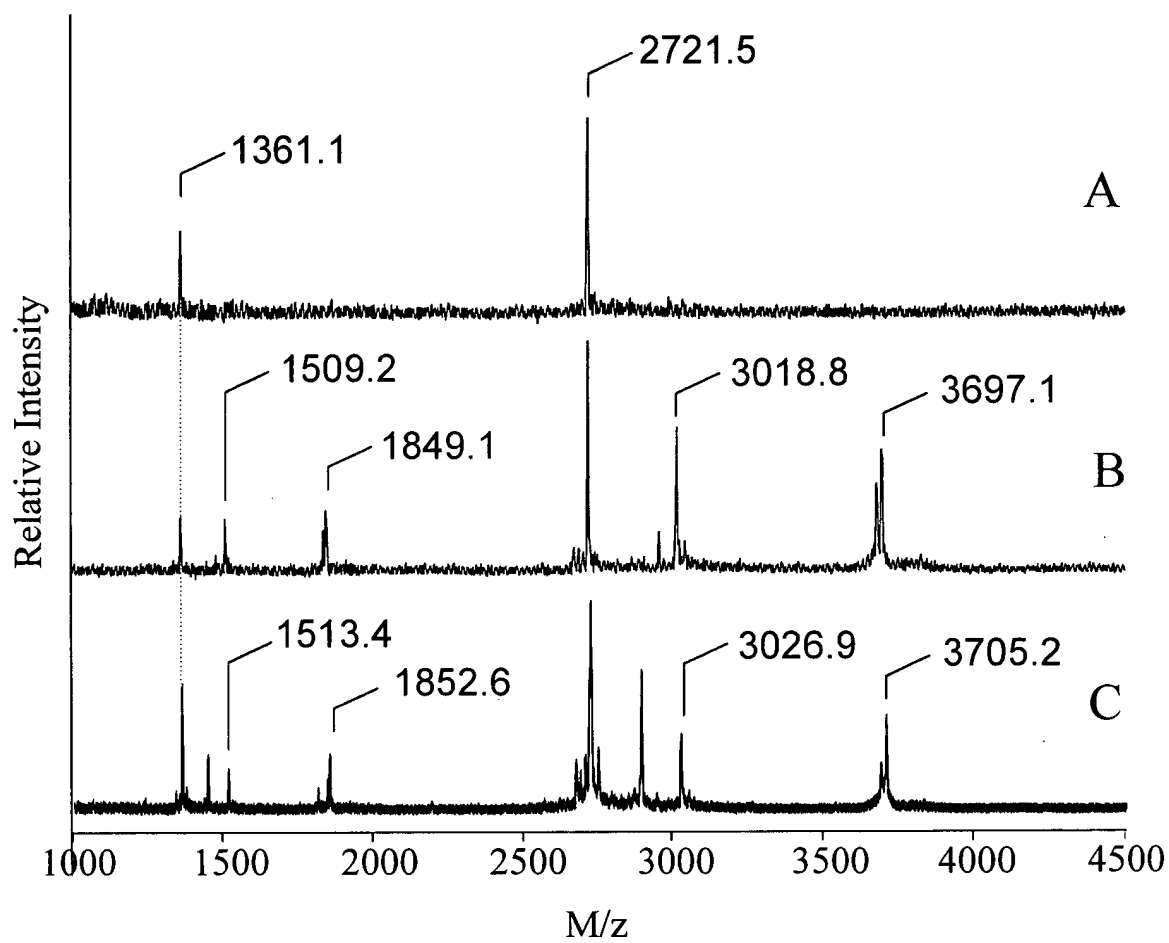


Figure 19

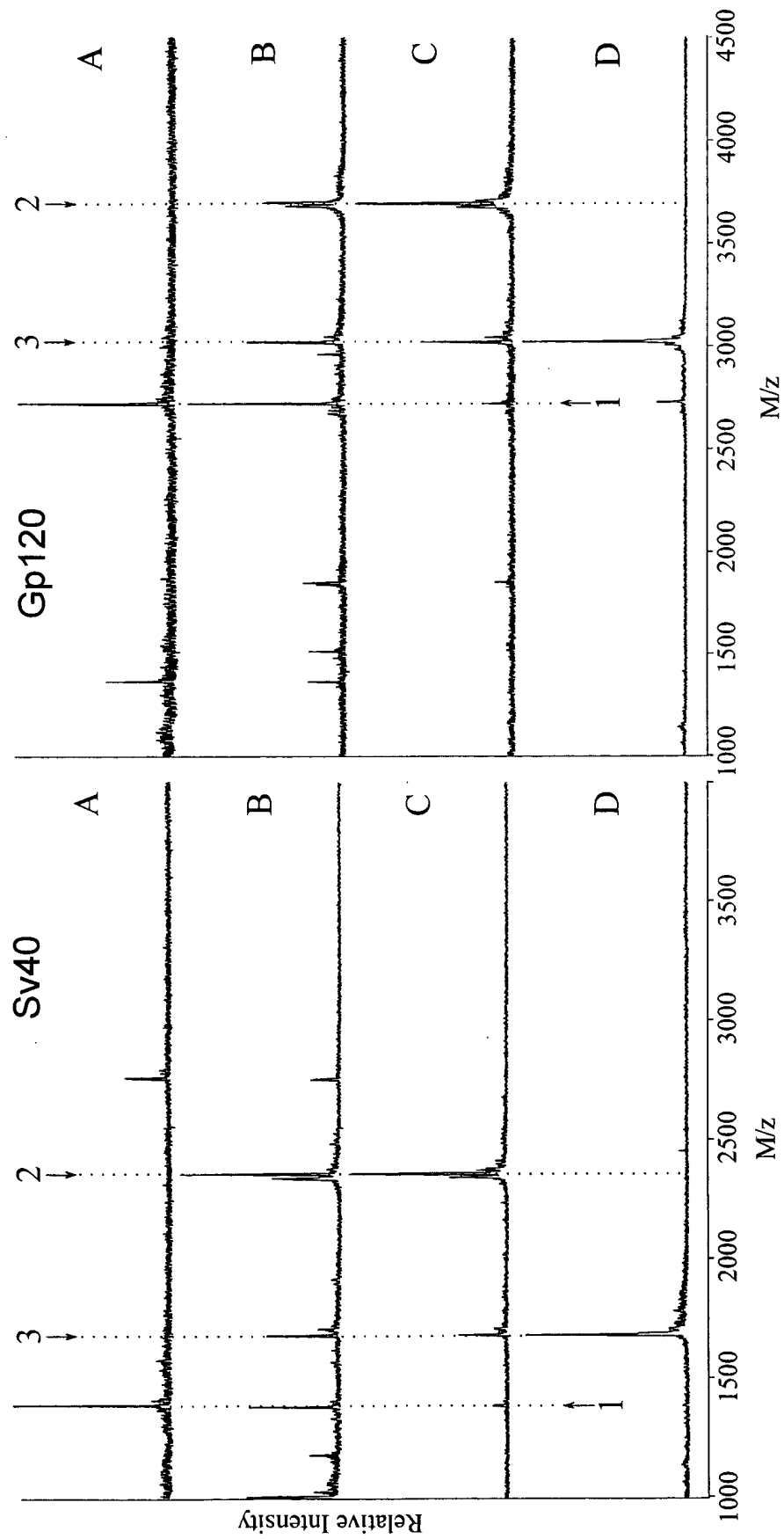


Figure 20

Analyte	Mass calculated (MH) ⁺	Mass measured (solution)	Mass measured (Streptavidin beads)
Reagent PC-ICAT#1	976.41 (uncleaved); 635.28 (cleaved)	976.94; 635.66	N/a
SV-40 peptide analog	1378.69; 2755.38 (homodimer)	2755.06 (homodimer); 1378.74;	N/a
SV-40 peptide analog+ PC-ICAT#1	2355.1 (uncleaved); 1676.0 (cleaved)	2755.53 (homodimer) 2355.36; 1675.98; 1378.36	Streptavidin beads: 2355.45; 1676.33; Photocleaved/supernatant: 1676.23
HIV gp120 peptide	2721.30	2721.52; 1361.14 (MH ₂) ⁺	N/a
HIV gp120 peptide + PC-ICAT#1	3697.71 (uncleaved) 3018.61 (cleaved)	3697.09; 3018.82; 2721.45; 1849.13 (uncleaved (MH ₂) ²⁺); 1509.18 (cleaved, MH ₂) ²⁺); 1360.88 (MH ₂) ⁺	Streptavidin beads: 3697.13; 3018.62; Cleaved/supernatant: 3018.89

Figure 21

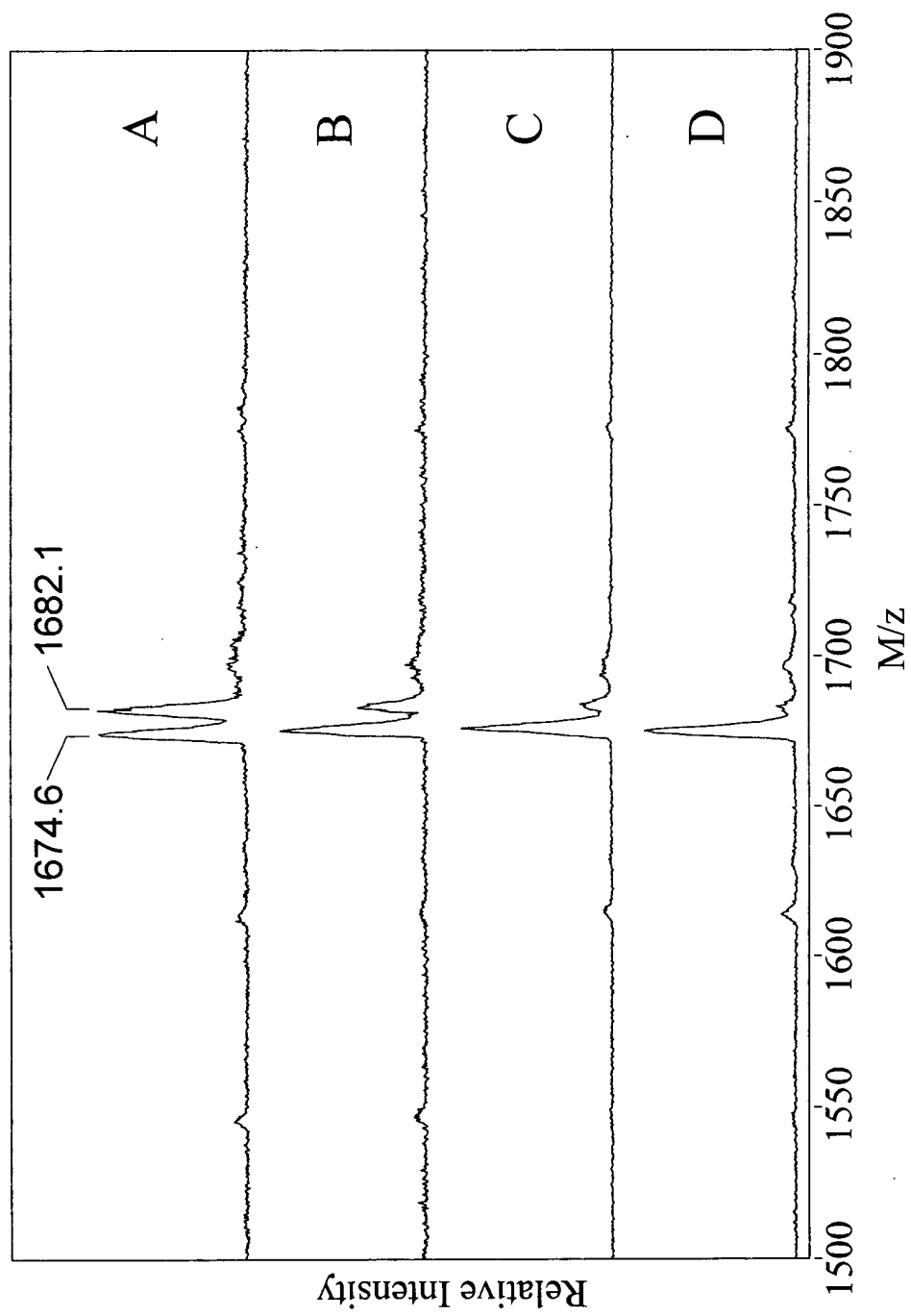


Figure 22

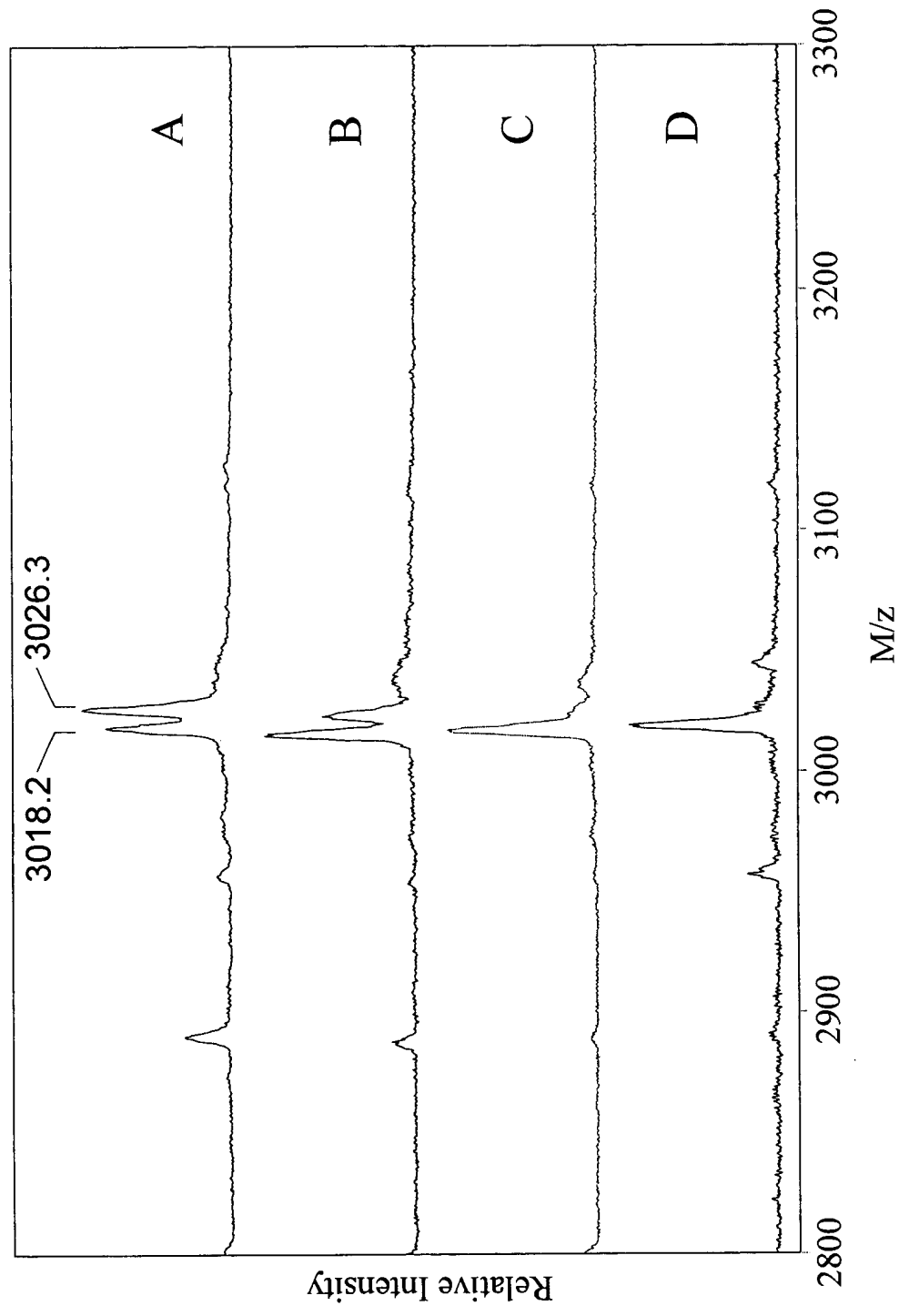


Figure 23A

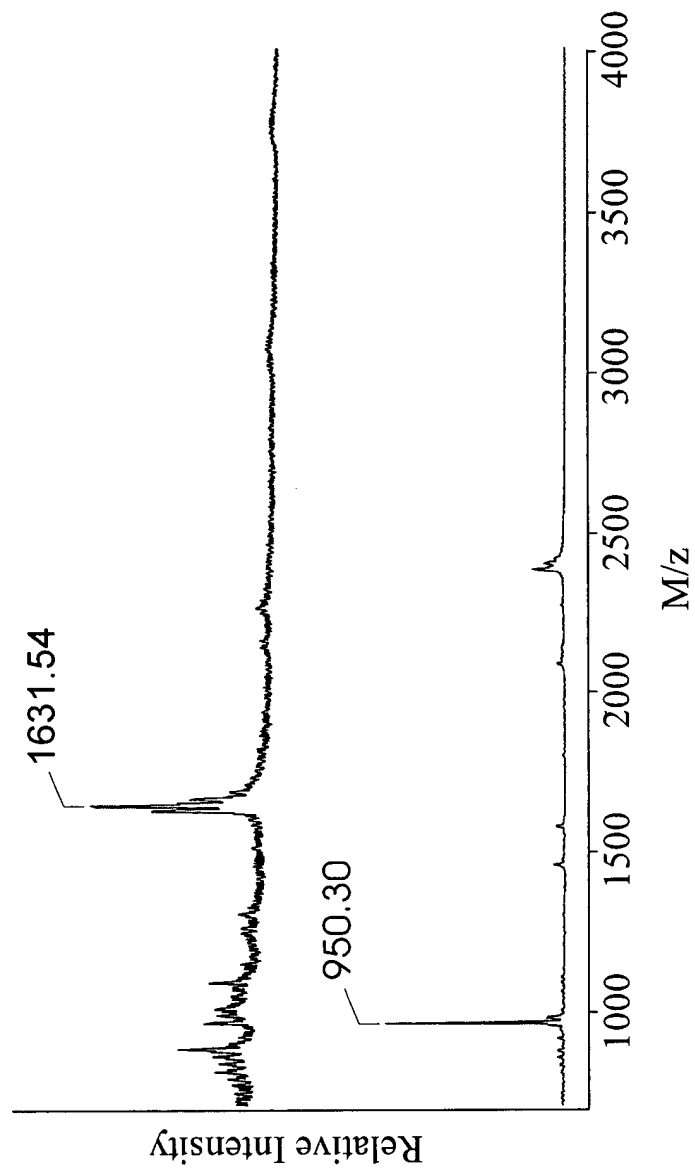


Figure 23B

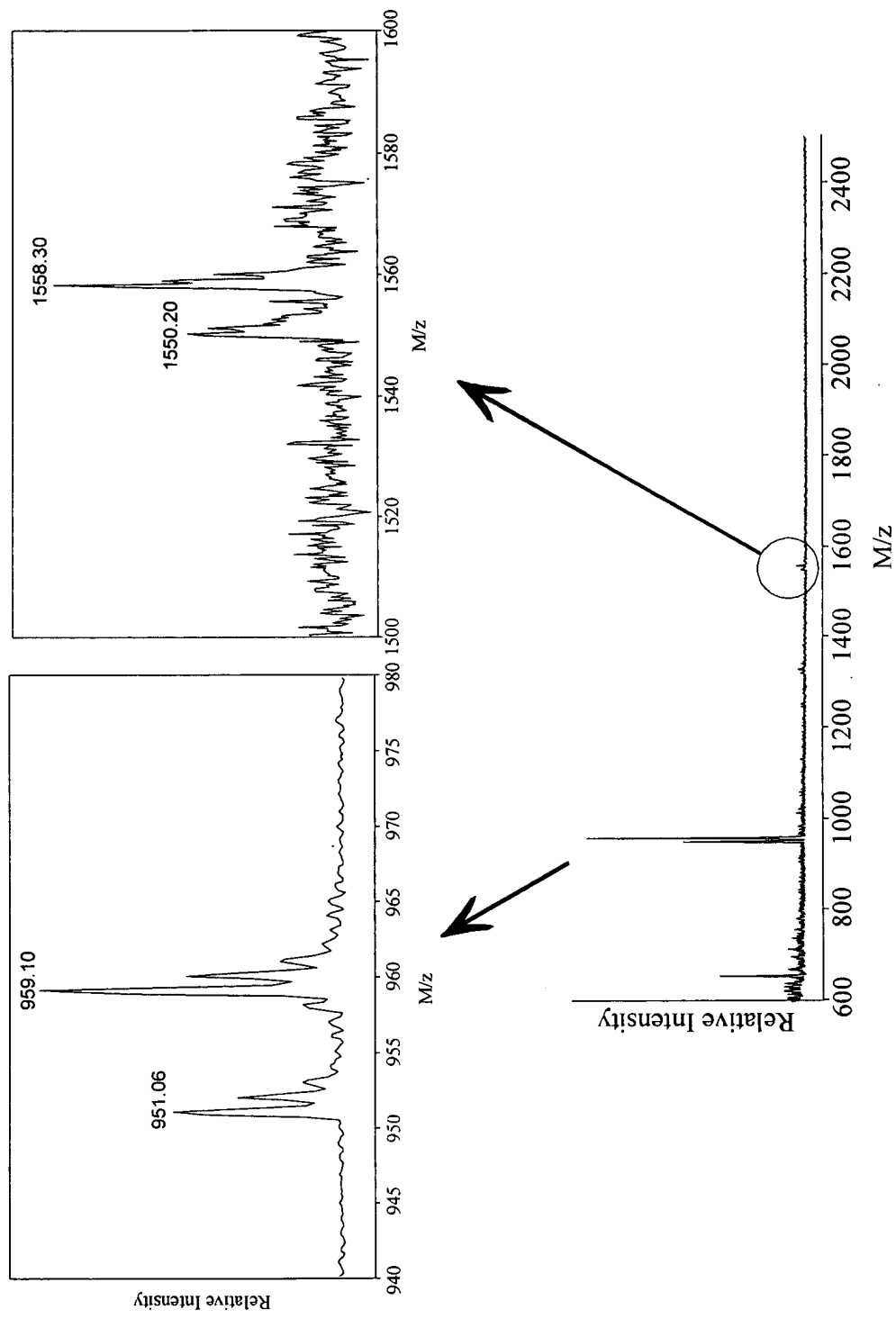


Figure 24

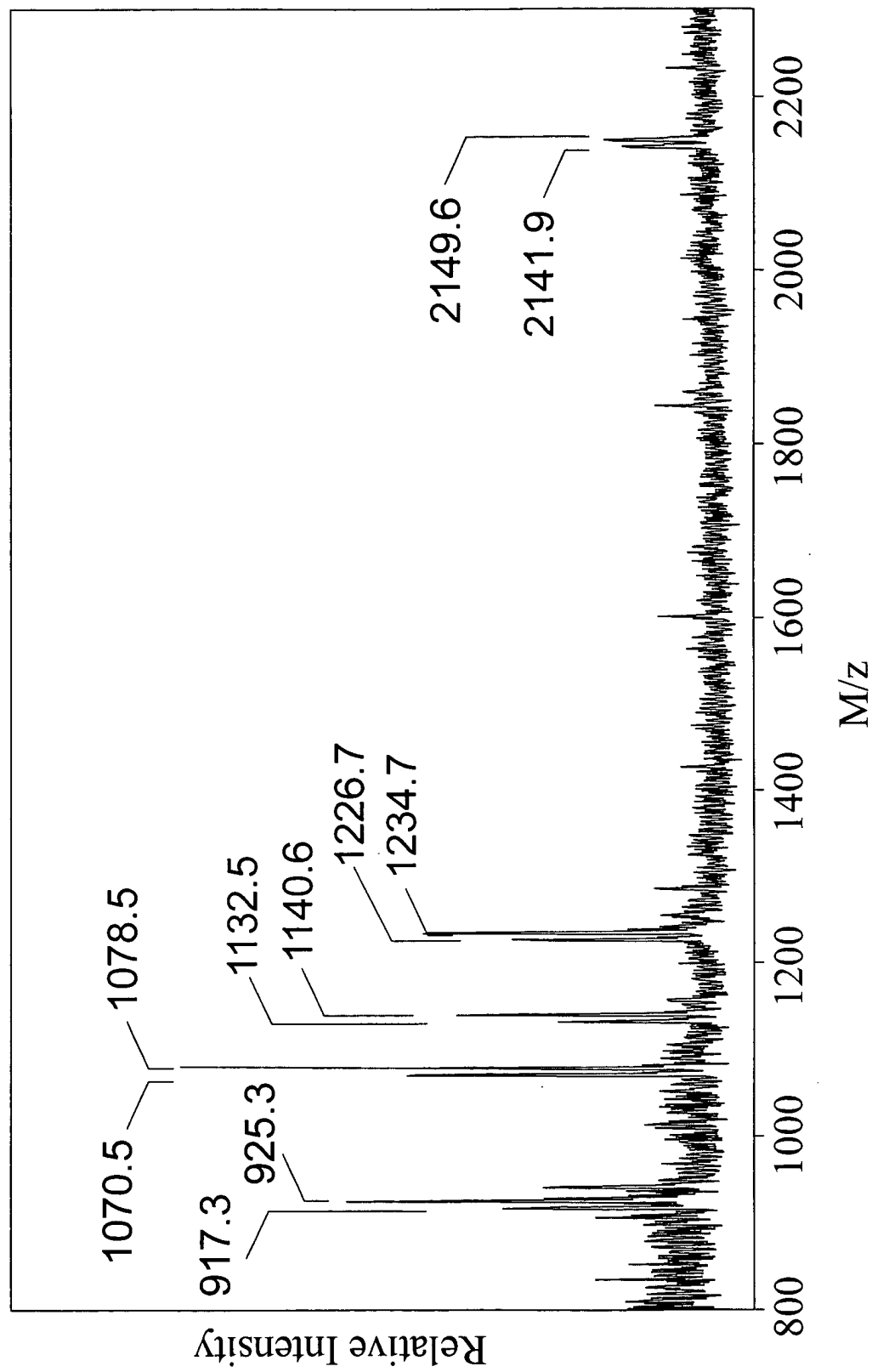


Figure 25

Peptide	H:L ratio (theory)	H:L ratio (experiment)	Standard Deviation	Error, [%]
HIV gp120	1	1.4898	0.126	48.0
	0.50	0.7838	0.038	56.0
	0.20	0.2403	0.004	20.0
	0.10	0.1579	0.043	9.0
SV40	1	1.0593	0.019	6.0
	2	1.9220	0.024	4.0
	5	3.7449	0.362	25.0
	10	5.7204	0.250	56.0

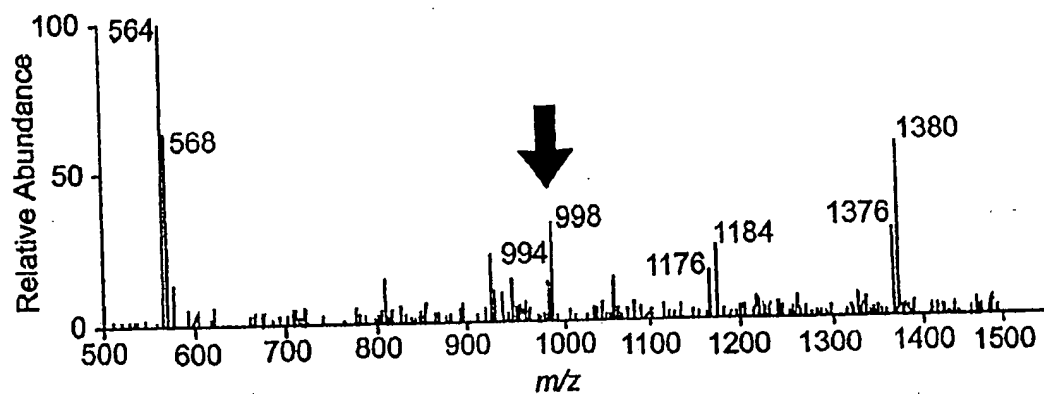


Figure 26A

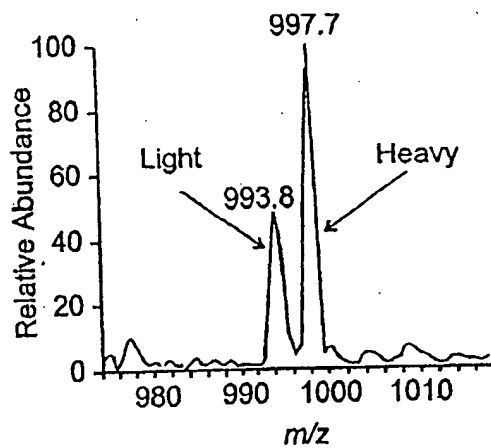


Figure 26B

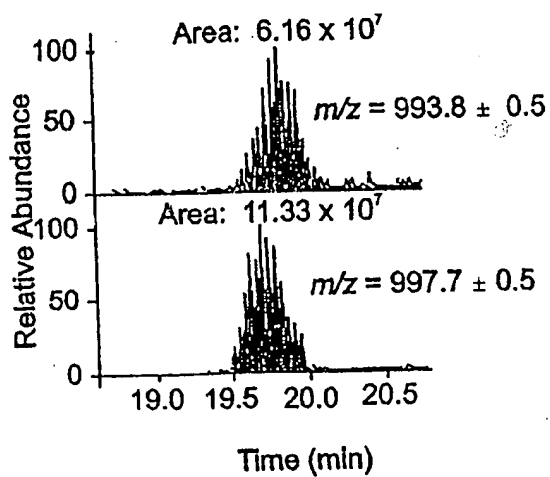


Figure 26C

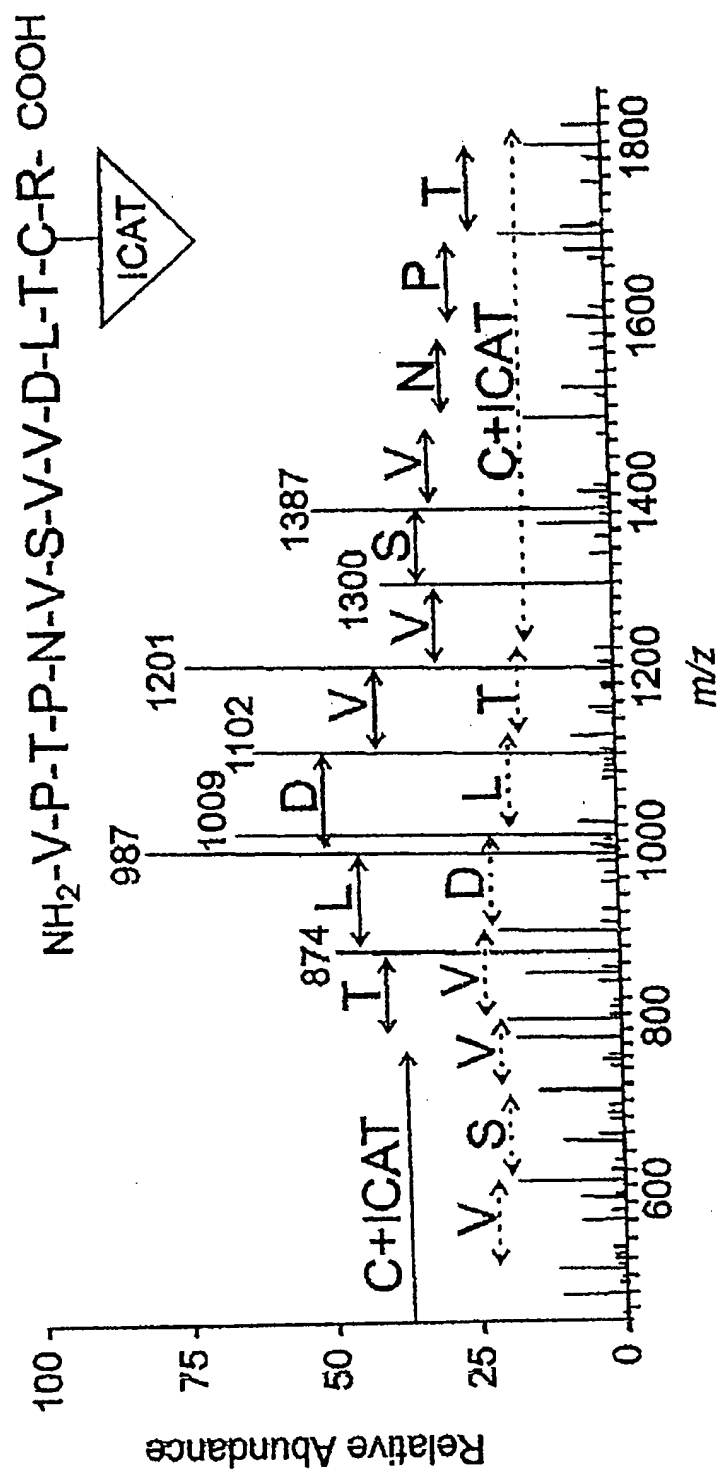


Figure 27A

#	<u>Rank/Sp</u>	<u>(M+H)⁺C*10⁴</u>	<u>Ions</u>	<u>Reference</u>	<u>Peptide</u>
1.	1/ 1	1994.3 4.4675	17/26	G3P_RABIT	(R)VPTPNVSVVDLTC#R (SEQ ID NO:60)
2.	2/ 403	1995.1 2.7366	13/34	SLTRNGL	(E)LGKPVLTANQVTIWEGLR (SEQ ID NO:61)
3.	3/ 3	1995.0 2.6591	16/36	FLP_LACCA	(N)LANPNVYTETLTAAATVCTI (SEQ ID NO:62)
4.	4/ 209	1995.0 2.6335	14/36	A42912	(Y)LALLPSDAEGPHGQFVTDK (SEQ ID NO:63)
5.	5/ 381	1995.1 2.4634	13/38	H69373	(L)ALLVLVAPAMAAAGNGEDLRN (SEQ ID NO:64)

Figure 2 7B

Figure 28

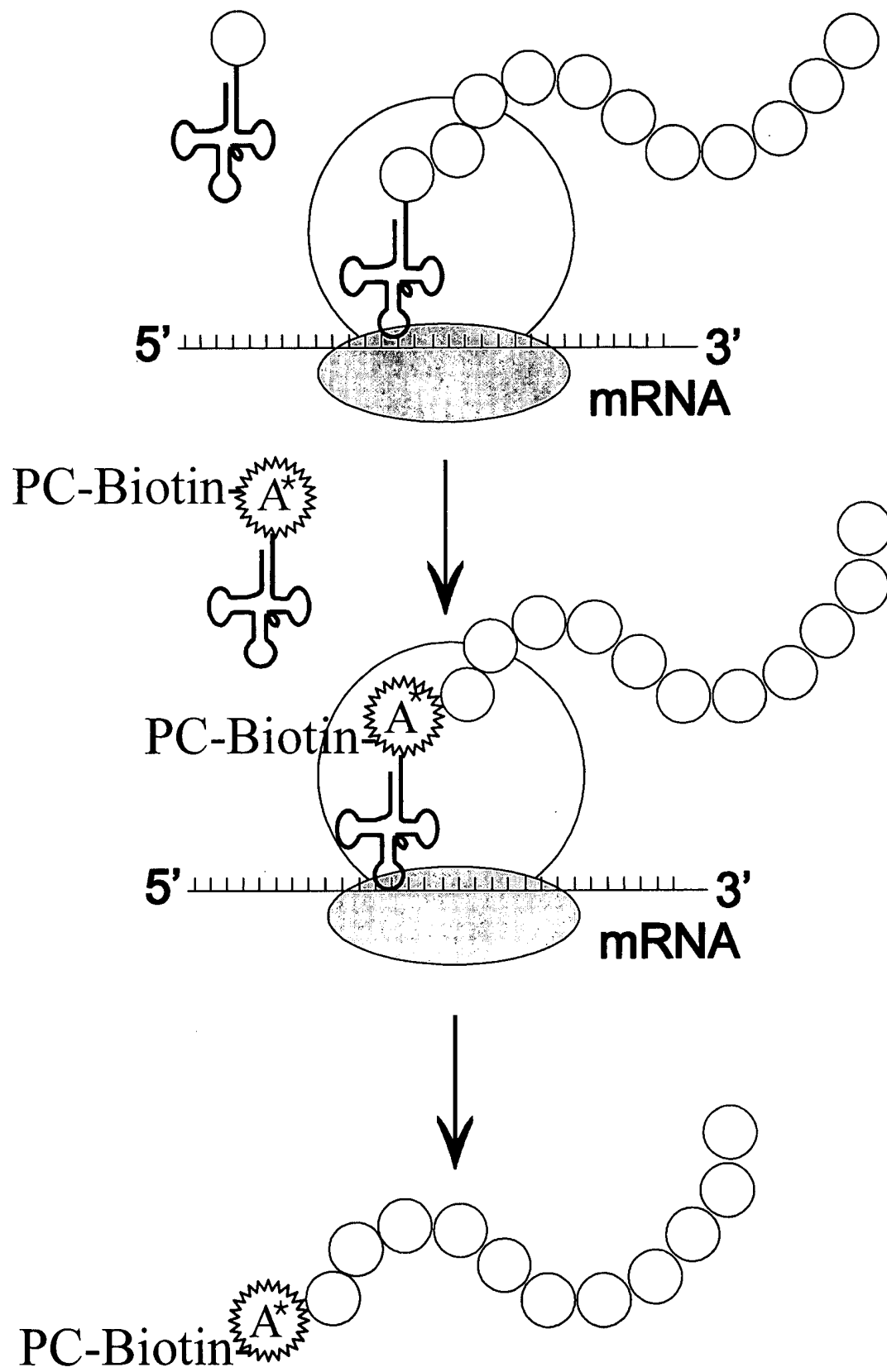


Figure 29

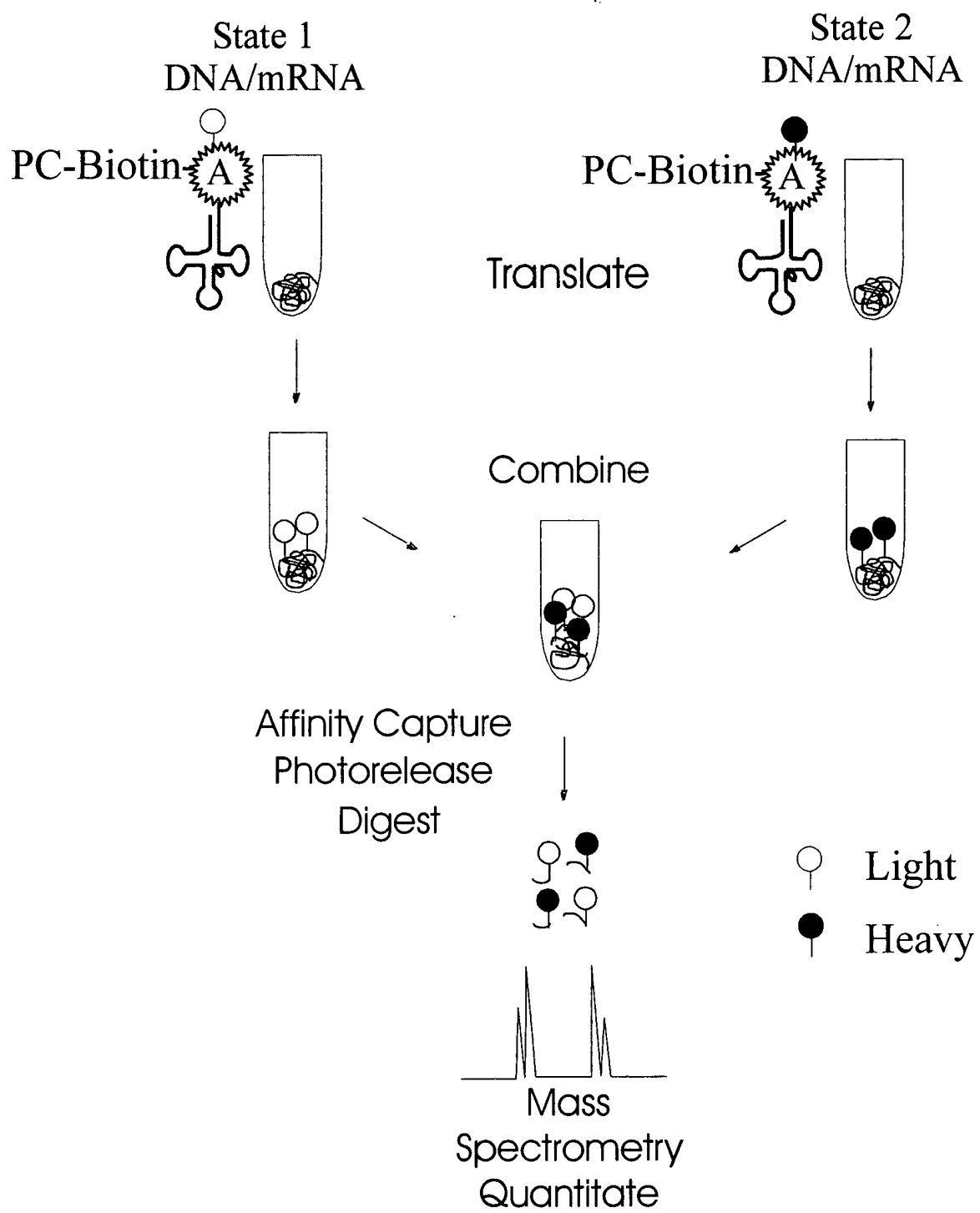


Figure 30

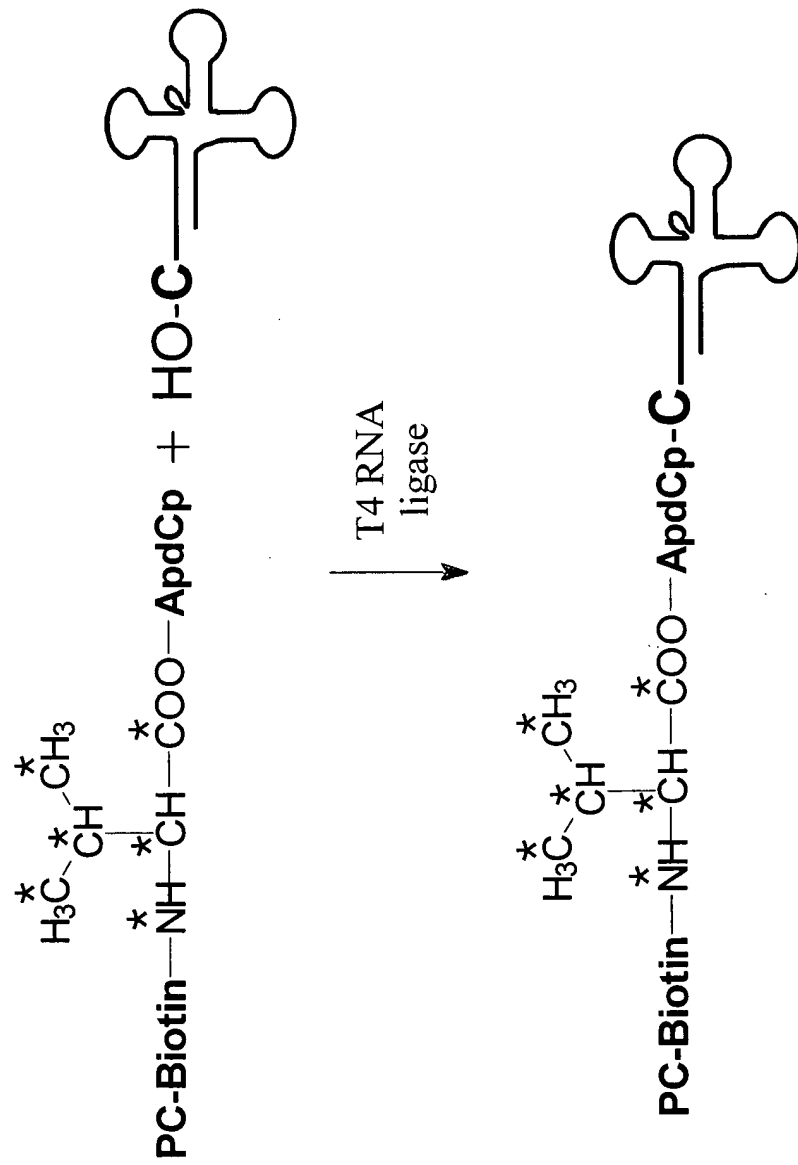


Figure 32

